NIH Symposium

RNA Biology 2015

March 11-12, 2015

PROGRAM AND ABSTRACT BOOK

Natcher Auditorium, NIH Campus
Bethesda, Maryland

INTERNAL USE ONLY

Welcome to the NIH Symposium *"RNA Biology 2015"*.

RNA biology has emerged as one of the most influential areas in modern biology and biomedicine.

Groundbreaking progress has been made over the last few decades in elucidating the basic mechanisms of RNA biogenesis and function. These efforts have in recent years been complemented by technological breakthroughs to determine RNA structures and the discovery of a wealth of, mostly unanticipated, new classes of RNAs, many still of unknown functions. RNAs have also emerged as the basis for some of the most powerful tools and technologies to manipulate biological functions and we have realized that RNA plays a role in most physiological and pathological events and as such is a prime target for, yet largely untapped, novel diagnostic, prognostic and therapeutic strategies.

Organized by the CCR Initiative in RNA Biology, this symposium brings together international leaders in the field of RNA Biology to take a snapshot of the state of the field and to explore the future of RNA biology. The symposium signals the NCI's commitment to the field of RNA biology and offers you an opportunity to learn more about the current status of RNA biology in development and disease, share your research, network with leading figures in the field, and to discuss the use and implications of these advances for clinical applications.

Sincerely,
The CCR Initiative in RNA Biology Steering Committee
Center for Cancer Research
National Cancer Institute

Tom Misteli, Ph.D., Symposium Chair
Susan Gottesman, Ph.D.
Stephen Hughes, Ph.D.
Javed Khan, M.D.
Stuart Le Grice, Ph.D.
Jeff Strathern, Ph.D.
Yun-Xing Wang, Ph.D.
Zhi-Ming (Thomas) Zheng, M.D., Ph.D.
Joseph Ziegelbauer, Ph.D.

NIH Symposium

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Wednesday, March 11, 2015

7:30 a.m. Registration opens

8:30 a.m. **Welcome**
Tom Misteli, Ph.D., National Cancer Institute

SESSION 1: RNA BIOGENESIS
Chair: *Tom Misteli, Ph.D., National Cancer Institute*

8:45 a.m. **"Single-molecule Imaging Reveals Switch Between Spurious and Functional ncRNA Transcription"**
Daniel Larson, Ph.D., National Cancer Institute

9:15 a.m. **"Following Single Molecules of mRNA in Living Cells"**
Robert Singer, Ph.D., Albert Einstein College of Medicine

9:45 a.m. **"SINEs of Anarchy: How Alu Elements Alter mRNA Metabolism"**
Lynne Maquat, Ph.D., University of Rochester Medical Center

10:15 a.m. Break

SESSION 2: RNA PROCESSING
Chair: *Shalini Oberdoerffer, Ph.D., National Cancer Institute*

10:30 a.m. **"Targeted Modulation of Alternative Splicing as a Therapeutic Strategy"**
Adrian Krainer, Ph.D., Cold Spring Harbor Laboratory

11:00 a.m. **"RNA Binding Proteins in EMT and Myotonic Dystrophy"**
Christopher Burge, Ph.D., Massachusetts Institute of Technology

11:30 a.m. **"mRNA Surveillance Starts on the Ribosome: Insights from Biochemistry and Profiling"**
Rachel Green, Ph.D., Howard Hughes Medical Institute and Johns Hopkins University School of Medicine

12:00 p.m. **LUNCH BREAK AND POSTER VIEWING**

SESSION 3: MicroRNAs
Chair: *Joseph Ziegelbauer, Ph.D., National Cancer Institute*

1:15 p.m. **"MicroRNA-based Therapeutics"**
Frank Slack, Ph.D., Harvard University

1:45 p.m. **"The Many Faces of Base Pairing Small RNAs"**
Gisela Storz, Ph.D., National Institute of Child Health and Human Development

2:15 p.m. **"Prospects for Riboswitch and Ribozyme Discovery"**
Ronald Breaker, Ph.D., Howard Hughes Medical Institute and Yale University

2:45 p.m. Break

SESSION 4: NIH DIRECTOR'S WEDNESDAY AFTERNOON LECTURE SERIES

3:00 p.m. *The Annual Margaret Pittman Lecture*
"CRISPR-Cas Genome Surveillance: From Basic Biology to Transformative Technology"
Jennifer Doudna, Ph.D., Howard Hughes Medical Institute and University of California, Berkeley

4:00 p.m. **POSTER SESSION WITH WALS RECEPTION**

6:00 p.m. Adjourn

Thursday, March 12, 2015

SESSION 5: NON-CODING RNAs
Chair: *Ashish Lal, Ph.D., National Cancer Institute*

8:30 a.m. **"Epigenetic Genome Control by RNA Processing Factors and Heterochromatin Machinery"**
Shiv Grewal, Ph.D., National Cancer Institute

9:00 a.m. **"Linking RNA to Cancer *In Vivo*"**
John Rinn, Ph.D., Harvard University and the Broad Institute

9:30 a.m. **"Genome Regulation by Long Non-coding RNAs"**
Howard Chang, M.D., Ph.D., Howard Hughes Medical Institute and Stanford University

10:00 a.m. Break

SESSION 6: RNA STRUCTURE
Chair*: Stuart Le Grice, Ph.D., National Cancer Institute*

10:15 a.m. **"Insights into the Structural Basis and Mechanism of HIV-1 Genome Packaging"**
Mike Summers, Ph.D., University of Maryland, Baltimore County

10:45 a.m. **"Structural Studies of Fluorescent RNA Mimics of GFP"**
Adrian Ferré-D'Amaré, Ph.D., National Heart, Lung, and Blood Institute

SESSION 7: RNA IN DISEASE

Chair*: Stuart Le Grice, Ph.D., National Cancer Institute*

11:15 a.m. **"Senescence Long Non-coding RNAs"**
Myriam Gorospe, Ph.D., National Institute on Aging

11:45 a.m. **"Regulation and Function of microRNAs in Physiology and Cancer"**
Joshua Mendell, M.D., Ph.D., University of Texas Southwestern Medical Center

12:15 p.m. **LUNCH BREAK AND POSTER SESSION**

SESSION 8: KEYNOTE ADDRESS

Chair: *Jeffrey Strathern, Ph.D., National Cancer Institute*

1:45 p.m. **"The Origins and Functions of Non-coding RNAs"**
Phillip Sharp, Ph.D., Massachusetts Institute of Technology

SESSION 9: RNA THERAPY

Chair: *Jeffery Strathern, Ph.D., National Cancer Institute*

2:45 p.m. **"Therapeutic Targeting of microRNAs"**
Sakari Kauppinen, M.Sc., Ph.D., Aalborg University

3:15 p.m. **"Optimizing RNAi Chemistry and Formulation for the Treatment of Neurodegenerative Disorders"**
Anastasia Khvovora, Ph.D., University of Massachusetts Medical Center

3:45 p.m. **"Developing RNAi Therapeutics for the Treatment of Rare Diseases"**
Rachel Meyers, Ph.D., Alnylam Pharmaceuticals, Inc.

4:15 p.m. Adjourn

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Speaker Abstracts

SINGLE-MOLECULE IMAGING REVEALS SWITCH BETWEEN SPURIOUS AND FUNCTIONAL ncRNA TRANSCRIPTION

Larson, D.
Laboratory of Receptor Biology and Gene Expression, Center for Cancer Research, NCI, NIH, Bethesda, MD

Novel imaging techniques have made it possible to quantitatively measure gene expression in living cells by directly observing fluorescently tagged RNA synthesis over time. Here, we use this single-molecule technique to study transcription kinetics of the *GAL10* locus, which is regulated by sugar availability. We show that transcription occurs in bursts of high activity followed by periods of inactivity, each lasting several minutes. This stochastic, punctate behavior results in 'noise' in gene expression and is not visible in population studies, which instead give the impression of a gradual response to sugar availability. To understand how bursts of *GAL10* transcription are regulated, we focused on the role of a non-coding RNA produced from the antisense strand. Genomic data indicates that eukaryotic genomes are ubiquitously transcribed, but the function of these RNAs is largely unknown. To elucidate how antisense expression controls *GAL10* transcriptional dynamics, sense and antisense RNA are visualized simultaneously using the MS2 and PP7 RNA labeling. We find that antisense RNA is only transiently present at the locus when it is being transcribed and is not maintained on chromatin to recruit repressive complexes or form R-loops. Furthermore, antisense transcription displays different behavior during repression and activation, suggesting multiple roles for non-coding RNA, even at the same locus.

TRANSLATION OF SINGLE MOLECULES OF mRNA IN LIVING CELLS

Singer, R.H.
Department of Anatomy and Structural Biology and Gruss Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY and Janelia Research Campus of the HHMI, Ashburn, VA

Imaging has been an essential tool to analyze the dynamic properties of RNA. New technologies in optical microscopy and RNA tagging methods allowed us to detect and track individual mRNA molecules in single living cells, yielding insights that could not have been obtained through any ensemble measurement: measuring the time for transcription elongation, nuclear pore transport and localization and degradation in the cytoplasm. We have been dedicated to developing and implementing these technologies to further the understanding of dynamics of mRNA by observing translational regulation of single mRNAs in living neural tissues of a transgenic mouse. To study translation at the single molecule level, we have engineered an mRNA translation reporter: the mRNAs are labeled by two fluorescent RNA binding proteins, one in the coding region that is knocked off by ribosome transit. The kinetics of first round of translation now tractable at the single molecule level *in vivo*.

Supported by NIH Grants to RH Singer and by the HHMI

SINEs OF ANARCHY: HOW ALU ELEMENTS ALTER mRNA METABOLISM

Maquat, L.E.[1,2]
[1]Department of Biochemistry & Biophysics, School of Medicine and Dentistry; [2]Center for RNA Biology, University of Rochester, Rochester, NY

Staufen1-mediated mRNA decay (SMD), which occurs when translation terminates sufficiently upstream of a STAU-binding site (SBS), is important to developmental and homeostatic pathways[3]. An SBS can be created by intramolecular base-pairing within an mRNA 3'UTR or by intermolecular base-pairing between a 3'UTR and one or more lncRNAs. Intermolecular base-pairing in humans involves Alu elements[4], which are a type of small interspersed repetitive element (SINE), whereas intermolecular base-pairing in rodents involves B and identifier SINEs[5]. Roles of STAU1 dimerization[6] and the STAU1 paralog STAU2[7] in SMD will be discussed. A mechanism by which mRNAs crosstalk in a way that involves direct mRNA−mRNA interactions between 3'UTR Alu elements in each mRNA will be described, uncovering a new role for mammalian-cell mRNAs[8]. This unexpected function, together with our discovering how STAU1 binding to inverted repeated 3'UTR Alu elements (IR*Alu*s) competes with nuclear retention mediated by $p54^{nrb}$ binding to 3'UTR IR*Alu*s and also the repression of cytoplasmic translation mediated by PKR binding to 3'UTR IR*Alu*s[9], adds new layers of complexity to the network of post-transcriptional interactions that regulate gene expression and involve ncRNA.

[3]Park E, Maquat LE (2013) *WIRES RNA* 4:423-35.
[4]Gong C, Maquat LE (2011) *Nature* 470: 284-8.
[5]Wang J, Gong C, Maquat LE (2013) *Genes Dev* 27: 793-804.
[6]Gleghorn ML, Gong C, Kielkopf CL, Maquat LE (2013) *NSMB* 20:515-24.
[7]Park E, Gleghorn ML, Maquat LE (2013) *PNAS* 110: 405-12.
[8]Gong, C., Tang, Y. and Maquat, L.E. (2013) *NSMB* 20:1214-20.
[9]Elbarbary, R., Li, W., Tian, B. and Maquat, L.E. (2013) *Genes Dev* 27:1495-1510.

TARGETED MODULATION OF ALTERNATIVE SPLICING AS A THERAPEUTIC STRATEGY

Krainer, A.R.
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

I will discuss targeted modulation of alternative splicing by antisense oligonucleotides (ASOs), and its application to understand and treat CNS disease, focusing on spinal muscular atrophy (SMA). SMA is a motor-neuron disease, caused by loss-of-function mutations in the Survival motor neuron 1 (SMN1) gene. Patients retain one or more copies of the nearly identical, but splicing-defective SMN2 gene. The small amount of full-length SMN expressed from SMN2 is essential for survival of SMA patients, but only partially compensates for the loss of SMN1. Together with Isis Pharmaceuticals, we developed ISIS-SMNRx, an ASO complementary to a region comprising a potent splicing silencer in intron 7. This ASO efficiently promotes SMN2 exon 7 inclusion and restores SMN protein levels in various tissues of SMA mouse models. I will present our data comparing CNS versus systemic delivery of ISIS-SMNRx in a severe SMA mouse model. Unexpectedly, our results indicate that SMA is not motor-neuron cell-autonomous, and suggest that correction of SMN2 splicing in peripheral tissues is necessary and perhaps sufficient for phenotypic rescue, at least in the context of the mouse model. I will also review the ongoing clinical trials of ISIS-SMNRx.

RNA BINDING PROTEINS IN EMT AND MYOTONIC DYSTROPHY

Katz, Y.[1], Wang, E.T. [2], and Burge, C.B.[2,3]
[1]Broad Institute of MIT and Harvard; [2]Koch Institute for Integrative Cancer Research;
[3]Department of Biology, Massachusetts Institute of Technology, Cambridge MA

RNA binding proteins (RBPs) play important roles in pathogenesis of several major human diseases, and may help to specify cell state. We are studying major families of RBPs including the conserved Musashi (Msi) family, the CUGBP1, Elav-like family (CELF), and Muscleblind-like (MBNL) family, and their roles in cell state transitions and disease. Msi proteins are expressed in stem/progenitor and cancer cells, but not in most differentiated cells, consistent with a role in cell state control. We have found that Msi genes are rarely mutated but frequently overexpressed in human cancers and are associated with an epithelial-luminal cell state. By analyses of the transcriptome and translatome of normal and Msi-depleted cells, we found that Msi proteins regulate translation of genes implicated in epithelial cell biology and epithelial-to-mesenchymal transition (EMT), and promote an epithelial splicing pattern. Overexpression of Msi proteins inhibited the translation of Jagged1, a factor required for EMT, and repressed EMT in cell culture and in mammary gland *in vivo*. Knockdown of Msis in epithelial cancer cells promoted loss of epithelial identity. Our results show that mammalian Msi proteins contribute to an epithelial gene expression program in neural and mammary cell types. RBPs of the CELF and MBNL families contribute to heart and skeletal muscle development and are implicated in myotonic dystrophy (DM), in which RNAs containing expanded CUG repeats accumulate, leading to sequestration of MBNL proteins and overexpression of CELF proteins. To understand genome-wide functions of these factors, we analyzed transcriptome dynamics following induction of *CELF1* or *CELF2* in adult mouse heart and of *CELF1* in muscle by RNA-seq, complemented by crosslinking/immunoprecipitation-sequencing (CLIP-seq) analysis of mouse cells and tissues to distinguish direct from indirect regulatory targets. We identified hundreds of mRNAs bound in their 3' UTRs by both CELF1 and by the developmentally-induced MBNL1 protein, a 3-fold greater overlap in target messages than expected, including messages involved in development and cell differentiation. The extent of 3' UTR binding by CELF1 and MBNL1 predicted the degree of mRNA repression or stabilization, respectively, following CELF1 induction. However, CELF1's RNA binding specificity *in vitro* was not detectably altered by coincubation with recombinant MBNL1. These findings support a model in which CELF and MBNL proteins bind independently to mRNAs but functionally compete to specify downregulation or localization/stabilization, respectively, of hundreds of mRNA targets.

Expression of many alternative 3' UTR isoforms was altered following CELF1 induction, with 3' UTR binding associated with downregulation of isoform and gene. The splicing of hundreds of alternative exons was oppositely regulated by these proteins, confirming an additional layer of regulatory antagonism previously observed in a handful of cases. The regulatory relationships between CELFs and MBNLs in control of both mRNA abundance and splicing appear to have evolved to enhance developmental transitions in major classes of heart and muscle genes. This extensive antagonism may exacerbate misregulation of gene and mRNA isoform expression in DM.

MECHANISMS OF mRNA SURVEILLANCE DEFINED BY RIBOSOME PROFILING AND BIOCHEMISTRY

Guydosh, N., Smith-Koutmos, K. and Green, R.
HHMI, Johns Hopkins University School of Medicine, Baltimore, MD

There are several mRNA surveillance pathways in eukaryotes that moderate the effects of natural errors in the cell and more broadly regulate gene expression. We have previously defined biochemical parameters of the factors Dom34, Hbs1 and Rli1 and their role on the ribosome in quality control system using our previously developed *in vitro* reconstituted yeast translation system. We are now beginning to define how poly-basic sequences lead to pausing during translation using *in vitro* reconstituted translation systems from *E. coli* and yeast. We also are conducting a broad analysis of the *in vivo* targets of mRNA surveillance (including NGD, NSD and NMD) using reporter constructs and genome wide ribosome profiling in yeast. The results from these approaches will be presented.

microRNA-BASED THERAPEUTICS IN CANCER

Slack, F.J.
Department of Pathology, BIDMC/Harvard Medical School, Boston, MA

MicroRNAs are small non-coding RNAs that regulate gene expression to control important aspects of development and metabolism such as cell differentiation, apoptosis and lifespan. *let-7* and miR-34 are microRNAs implicated in human cancer. Specifically, human *let-7* and miR-34 are poorly expressed or deleted in lung cancer, and over-expression of *let-7* or miR-34 in lung cancer cells inhibits their growth, demonstrating a role for these miRNAs as tumor suppressors in lung tissue. *let-7* and miR-34 regulate the expression of important oncogenes implicated in lung cancer, suggesting a mechanism for their involvement in cancer. We are focused on the role of these genes and other oncomiRs in regulating proto-oncogene expression during development and cancer, and on using miRNAs to suppress tumorigenesis.

THE MANY FACES OF BASE PAIRING SMALL RNAs

Updegrove, T.B.[1], Thomason, M.K.[1], Zhang A.[1], Schu, D.J.[2], Gottesman, S.[2], and Storz, G.[1]
[1]Cell Biology and Metabolism Program, NICHD, NIH, Bethesda, MD; [2]Laboratory of Molecular Biology, Center for Cancer Research, NCI, NIH, Bethesda, MD

Small RNAs (sRNAs) that base pair with one or more target mRNAs to regulate their translation and stability are critical to multiple stress responses in bacteria. In *E. coli* and many other organisms, Hfq, a ring-shaped homohexamer, is required to facilitate the limited base pairing between the sRNAs and their targets. For many years, Hfq-binding sRNAs were considered to be relatively similar, but a number of recent observations suggest that this is not the case and that the categorization of sRNAs is becoming increasingly difficult. First, Hfq has at least two distinct ways in which it interacts with sRNAs; these different binding properties have strong effects on the stability of the sRNA, the sequence requirements of regulated mRNAs and may correlate with different cellular roles with Class I sRNAs acting as emergency responders and Class II sRNAs acting as silencers. Second, it can no longer be assumed that base pairing sRNAs act solely by this one mechanism. While the McaS RNA of *Escherichia coli* base pairs with the mRNAs encoding master transcription regulators of curli and flagella synthesis, the small RNA activates synthesis of an exopolysaccharide by binding and titrating the global RNA-binding protein CsrA away from the mRNA encoding the biosynthetic enzymes. Third, while all initial focus was on sRNAs encoded in intergenic regions, more and more sRNA derived from the 3' end of protein coding genes are being discovered. The MicL RNA, which base pairs with the mRNA encoding the most abundant protein in the cell, is transcribed from a promoter located within the coding sequence of the *cutC* gene, and the copper sensitivity phenotype previously ascribed to inactivation of the *cutC* gene is actually derived from the loss of MicL. This raises the possibility that other phenotypes currently attributed to protein defects are due to deficiencies in unappreciated sRNAs.

PROSPECTS FOR RIBOSWITCH AND RIBOZYME DISCOVERY

Breaker, R.
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT

With the emergence of large genomic sequence data sets and the development of efficient computer algorithms for comparative sequence analysis, the discovery of novel structured RNAs has increased in recent years. For example, approximately 35 classes of riboswitches have been discovered that control gene expression when triggered by the selective binding of small molecules or ions. Similarly, a variety of natural ribozymes have been reported that catalyze various RNA processing reactions. Given that modern cells likely descended from ancient RNA World organisms that employed a great diversity of ribozymes, it is possible that far more types of non-coding RNAs might still await discovery.

Ongoing bioinformatics analyses are continuing to reveal the existence of highly-structured RNAs of unknown function. Some of these newly-found RNA motifs have characteristics suggesting they might function as riboswitches, whereas others appear to be excellent ribozyme candidates. In rare instances, large and highly-conserved non-coding RNAs are identified. We speculate that some of these RNAs are ribozymes that have novel biochemical functions. In this presentation, I will summarize some of the most recent riboswitch and ribozyme discoveries, and highlight the prospects for future discoveries.

CRISPR-CAS GENOME SURVEILLANCE: FROM BASIC BIOLOGY TO TRANSFORMATIVE TECHNOLOGY

Doudna, J.A.
Department of Molecular & Cell Biology, Department of Chemistry, Howard Hughes Medical Institute, University of California, Berkeley, CA; Physical Biosciences Division, Lawrence Berkeley National Lab, Berkeley, CA

The advent of facile genome engineering using the bacterial RNA-guided CRISPR-Cas9 system in animals and plants is transforming biology. I will present a brief history of CRISPR biology from its initial discovery through the elucidation of the CRISPR-Cas9 enzyme mechanism, providing the foundation for remarkable developments using this technology to modify, regulate or mark genomic loci in a wide variety of cells and organisms. These results highlight a new era in which genomic manipulation is no longer a bottleneck to experiments, paving the way to both fundamental discoveries in biology, with applications in all branches of biotechnology, and strategies for human therapeutics. Recent results regarding the molecular mechanism of Cas9 and its use for targeted cell-based therapies will be discussed.

EPIGENETIC GENOME CONTROL BY RNA PROCESSING FACTORS AND HETEROCHROMATIN MACHINERY

Grewal, S.
Laboratory of Biochemistry and Molecular Biology, Center for Cancer Research, NCI, NIH, Bethesda, MD

The regulation of protein-coding and non-coding RNAs is linked to nuclear processes including chromatin modifications and gene silencing. However, the mechanisms that distinguish RNAs and mediate their functions are poorly understood. We describe a nuclear RNA processing network in fission yeast with a core module comprising the Mtr4-like protein, Mtl1, and the zinc finger protein, Red1. The Mtl1-Red1 core promotes degradation of mRNAs and non-coding RNAs, and associates with different proteins to assemble heterochromatin via distinct mechanisms. Mtl1 also forms Red1-independent interactions with evolutionarily conserved proteins named Nrl1 and Ctr1, which associate with splicing factors. Whereas Nrl1 targets transcripts with cryptic introns to form heterochromatin at developmental genes and retrotransposons, Ctr1 functions in processing intron-containing telomerase RNA. Together with our discovery of widespread cryptic introns, including in non-coding RNAs, these findings reveal unique cellular strategies for recognizing regulatory RNAs and coordinating their functions in response to developmental and environmental cues.

LINKING RNA TO CANCER *IN VIVO*

Sauvageau, M.[1,2], Clohess, S.[2], Pandolfi, P.P.[2], and Rinn, J.[1,2]
[1]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA; [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Recent evidence point to a role for long non-coding RNAs (lncRNAs) as key regulatory factors across diverse aspects of biology, including cancer pathways and pathogenesis. However, how lncRNAs contribute to the establishment or maintenance of cancer *in vivo* remains poorly understood. Moreover, we lack a comprehensive understanding of lncRNAs as diagnostic biomarkers and roles in human cancer models. Thus there is a critical need to put in place a platform for discovery that allows for systematic characterization of lncRNAs in cancer. Recently, we have begun to bridge this gap using a systematic approach to analyzing the wealth of existing cancer mutation and aberrant gene expression data, with the objective to develop a platform that can be used to rapidly characterize the role and function of lncRNAs in cancer. By combining this data with existing gain- and loss-of function mutation lncRNA mouse models our team has generated, we have identified two top candidates: an oncogenic (*CRNDE*) and a tumor suppressor (*TUG1*) lncRNA. Using these candidates to develop the platform, we aim to comprehensively and systematically test the roles of lncRNAs *in vivo* using multiple existing genetic models and cancer backgrounds.

GENOME REGULATION BY LONG NON-CODING RNAs

Chang, H.Y.
Center for Personal Dynamic Regulomes, HHMI, the Program in Epithelial Biology and Cancer Biology, Stanford University School of Medicine, Stanford, CA

The discovery of extensive transcription of long non-coding RNAs (lncRNAs) provide an important new perspective on the centrality of RNA in gene regulation. I will discuss genome-scale strategies to discover and characterize lncRNAs. Genome-wide mapping of RNA secondary structures, termed the structurome, provides important clues to potential functions of regulatory RNAs. An emerging theme from multiple model systems is that lncRNAs form extensive networks of ribonucleoprotein (RNP) complexes with numerous chromatin regulators, and target these enzymatic activities to appropriate locations in the genome. Consistent with this notion, long non-coding RNAs can function as modular scaffolds to specify higher order organization in RNP complexes and in chromatin states. The importance of these modes of regulation is underscored by the newly recognized roles of long RNAs in developmental patterning and cancer.

NMR METHODS FOR LARGE RNAs

Summers, M.F.[1], Keane, S.[1], Heng, X.[1], Lu, K.[1], Garyu, L.[1], Monti, S.[1], Barton, S.[1], LaCotti, C.[1], Dorjsuren, B.[1], Kulandaivel, G.[1], Divakaruni, S.[1], Edme, K.[1], Jones, S.[1], Hiremath, A.[1], Tummillo, D.[1], Hosic, A.[1], Albrecht, S.[1], Garcia, E.[2], Kharytonchyk, S.[2], Telesnitsky, A.[2], Johnson, B.[1,3], and Chiu, W.[4]

[1]Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, MD; [2]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI; [3]One Moon Scientific, Inc., Westfield, NJ; [4]National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX

NMR is a powerful tool for probing RNA structure, but its application to larger RNAs is complicated by a number of factors, particularly limited chemical shift dispersion. We have developed a suite of 2H-edited NMR approaches and applied it to the intact HIV-1 5′-leader -- a 356 nucleotide RNA that is packaged into virions as a 712 nucleotide dimer. In some cases, structural elements could be identified from 2D 1H NMR NOESY spectra obtained for RNAs prepared with different combinations of nucleotide- and atom-specific 2H substitutions. Assignments were facilitated by analysis of 2D NOESY spectra obtained for differentially labeled, annealed RNA fragments. In addition, elements with signals in crowded regions of the 2H-edited spectra were identified using an approach that involves replacement of a short stretch of adjacent base pairs by A-U base pairs (long-range probing by adenosine interaction detection, or "lr-AID"). NMR signal assignments and assignment validation were facilitated by a ^{1}H NMR chemical shift database approach. A summary of these new approaches, and progress toward determination of the 3D structure of the HIV-1 5′-leader, will be presented.

CRYSTAL STRUCTURE OF AN RNA MIMIC OF GFP REVEALS A G-QUADRUPLEX CORE

Warner, K.D., and Ferré-D'Amaré, A.R.
Laboratory of RNA Biophysics and Cellular Physiology, Biochemistry and Biophysics Center, National Heart, Lung and Blood Institute, NIH, Bethesda, MD

Green fluorescent protein (GFP) and its derivatives revolutionized the study of proteins. 'Spinach' is a recently reported *in vitro* evolved RNA mimic of GFP, which as genetically encoded fusions, makes possible live-cell, real-time imaging of biological RNAs, without resorting to large RNA-binding protein-GFP fusions (*1*). To elucidate the molecular basis of Spinach fluorescence, we have solved its co-crystal structure bound to its cognate exogenous chromophore, revealing that Spinach activates the small molecule by immobilizing it between a base triple, a G-quadruplex, and an unpaired guanine. Mutational and NMR analyses indicate that the G-quadruplex is essential for Spinach fluorescence, is also present in other fluorogenic RNAs, and may represent a general strategy for RNAs to induce fluorescence of chromophores. The structure has guided the design of a miniaturized 'Baby Spinach', and provides the foundation for structure-driven design and tuning of fluorescent RNAs (*2*).

K.D.W. was an NIH-Oxford-Cambridge Research Scholar. This work was supported in part by the Intramural Program of the National Heart, Lung and Blood Institute, NIH.

(*1*) Paige, J.S., Wu, K.Y., & Jaffrey, S.R. *Science* 333, 643-646 (2011).
(*2*) Warner, K.D., Chen, M.C., Song, W., Strack, R.L., Thorn, A., Jaffrey, S.R., & Ferré-D'Amaré, A.R. *Nature Struct. Mol. Biol.* 21, 658-663 (2014).

SENESCENCE LONG NON-CODING RNAS

Abdelmohsen, K., Yoon, J.H., Martindale, J.L., Yang, X., Panda, A., Grammatikakis, I., Noh, J.H., Kim, J., Kim, K.M., Li, H., and Gorospe, M.
RNA Regulation Section, Laboratory of Genetics, National Institute on Aging, NIH, Baltimore, MD

Senescent cells accumulate in aging tissues, and their metabolic and gene expression profiles are linked to many age-related pathologies, including cancer. I discuss our recent studies on the expression patterns and functions senescence-associated long non-coding RNAs (lncRNAs), focusing on three lncRNAs differentially expressed with senescence: *LincRNA-p21* (Yoon et al., *Mol Cell*, 2012), which suppresses translation of select mRNAs, *HOTAIR* (Yoon et al., *Nat Commun*, 2013), which promotes ubiquitin-mediated proteolysis of select proteins, and *7SL* (Abdelmohsen et al., *Nuc Acids Res*, 2014) which suppresses p53 translation.

REGULATION AND FUNCTION OF NON-CODING RNAs IN MAMMALIAN PHYSIOLOGY AND CANCER

Mendell, J.T.
Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX

Gain- and loss-of-function of non-coding RNAs can potently influence cellular behavior in normal physiologic states and in diseases such as cancer. In particular, dysregulation of microRNA (miRNA) expression has been clearly shown to result in dramatic phenotypic consequences. We previously demonstrated extensive control of miRNA expression by well-characterized oncogenic and tumor suppressor networks including the MYC, KRAS, and p53 pathways. More recently, we have uncovered germline and somatic mutations in core miRNA processing components such as DROSHA and DICER in tumors. The analysis of novel mouse models with gain- and loss- of miRNA function has revealed potent, and often unexpected, consequences of dysregulated miRNA expression and activity *in vivo*. More recently, we have applied these same approaches to dissect novel mechanisms of long non-coding RNA (lncRNA) regulation and function in mammalian physiology and cancer. I will present our latest results related to the regulation and function of non-coding RNAs and how these findings may be exploited for the development of novel therapeutic approaches.

THERAPEUTIC TARGETING OF microRNAs

Kauppinen, S.[1,2]
[1]Department of Clinical Medicine, Aalborg University; [2]Department of Haematology, Aalborg University Hospital, Copenhagen, Denmark

MicroRNAs (miRNAs) are a class of short endogenous non-coding RNAs that function as important post-transcriptional regulators of gene expression in many cellular and developmental processes. Furthermore, emerging evidence implies that miRNA dysregulation is prevalent and associated with the pathogenesis and progression of a wide variety of human diseases, including cancer, cardiovascular diseases, CNS disorders, inflammation, metabolic disorders and viral infections. Thus, miRNAs have emerged as a new class of promising targets for therapeutic intervention. I will provide an overview of the current strategies for inhibition of miRNAs and miRNA families using chemically modified antimiR oligonucleotides and describe recent advances in the discovery and development of miRNA-based therapeutics for the treatment of cardiometabolic disease and hepatitis C virus infection.

DEVELOPING RNA THERAPEUTICS FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS

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Some of the major obstacles to the development of oligonucleotide therapeutics (ONTs) for the treatment of non-liver diseases are inefficient tissue delivery and distribution and non-productive entrapment of internalized compounds in "oligonucleotide" sinks. Recent progress on several approaches toward improving biological efficacy of therapeutic oligonucleotides will be discussed including:

- Chemically modulated brain delivery and silencing with huntingtin gene as a model target
- Use of small molecule screening to expand the chemical diversity of therapeutic oligonucleotides
- Exploring exosomes as "native" shuttles for therapeutic oligonucleotide delivery

DEVELOPING RNAi THERAPEUTICS FOR THE TREATMENT OF RARE DISEASES

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Significant progress in delivery has fuelled the advancement of RNAi therapeutics as a promising new class of investigational medicines. For systemic, liver-directed delivery of RNAi therapeutics, we utilize a receptor targeting strategy, which is based on the covalent attachment of a synthetic multivalent N-acetylgalactosamine (GalNAc) ligand to siRNA. The ligand binds specifically to the asialoglycoprotein receptor (ASGPR), a hepatocellular transmembrane glycoprotein, which plays a key role in binding and rapid removal of desialylated glycoproteins from circulation. Subcutaneous (SC) administration of siRNA GalNAc conjugates results in robust RNAi-mediated gene silencing in the liver. We have optimized this platform to enable specific and potent knockdown of a large number of therapeutically relevant targets. We have validated this platform in human clinical trials across several rare diseases. The progress in this platform, and its clinical translation, will be discussed.

Poster Abstracts

P-1

DEVELOPMENT AND EVALUATION OF THIENOPYRIDINE COMPOUNDS AGAINST HIV-1 TAR

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HIV-1 trans-activation response (TAR) element RNA, a 59 bp stem-loop structure located at the 5'-end of the nascent viral transcript, is a cis-acting regulator of HIV transcription and has been considered a target for inhibiting virus replication. Known TAR binders are generally highly charged compounds or aminoglycosides that lack selectivity. In an attempt to find drug-like small molecules that bind to TAR, we utilized a small molecule microarray screening approach. This work resulted in the identification of a novel thienopyridine scaffold that binds to TAR, but not several other control oligonucleotides. Here, we describe continuing efforts to develop this class of TAR-binding compounds. A focused library of analogs was synthesized and evaluated in several complementary biochemical, biophysical and cell-based assays to study structure-activity relationship as well as understand the mode of action of this class of analogs. SHAPE probing is also used to map compound interactions directly in the context of a larger, more complex RNA. The results from this study are presented and insights on the structural determinants of binding of this class of compounds to TAR are discussed.

P-2

DEVELOPMENT OF NOVEL RIBONUCLEASES FOR SEQUENCING CHEMICAL MODIFICATIONS IN RNA

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Discovery and quantification of chemical modifications in cellular RNA requires a concerted effort through a combination of biochemical and analytical technologies. Here, we demonstrate the utilization of novel nucleoside specific ribonucleases to sequence the location of chemical modifications in RNA. We have made significant progress for large-scale overexpression and purification of plant-based U-specific and C-specific ribonucleases that aid in characterization of nucleoside modifications in RNA. The RNA cleavage properties, nucleoside specificity and the substrate recognition behavior towards modified nucleosides are systematically characterized by the state-of the art liquid chromatography coupled with mass spectrometry. We demonstrate the utility of this technology, analogous to the 4-lane DNA sequencing, by obtaining the complete sequence coverage and mapping of nucleoside modifications of commercially available tRNA(s) sample. We are confident that this technology will enable us to accomplish site-specific quantification of the modification levels unambiguously either for small RNA (tRNA, miRNA, etc) or large RNA (rRNA) in response to the exposure of cell to a variety of environmental insults.

P-3

SRSF3 AND HNRNP A1 REGULATE ALTERNATIVE RNA SPLICING AND GENE EXPRESSION OF HUMAN PAPILLOMAVIRUS 18 BY BINDING TWO FUNCTIONALLY DISTINGUISHABLE RNA CIS-ELEMENTS

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Human papillomavirus (HPV) is a double-stranded DNA tumor virus associated with cervical, anogenital and oropharyngeal cancers. HPV genome transcribes two major polycistronic pre-mRNAs (one early and one late) and hijack host cell splicing machinery for their alternative RNA splicing and gene expression. Thus, alternative splicing of the viral polycistronic pre-mRNAs regulates the expression of every viral protein, including E1, E2, E1^E4, E5, E6, E7, E6^E7, E2^E8, L1 and L2. In this study by using an *in vitro* RNA splicing assay and an *in vivo* RNA splicing reporter assay, we functionally characterized RNA splicing cis-elements and trans-acting splicing factors in regulation of the alternative RNA splicing and gene expression of HPV18, the second most prevalent oncogenic HPV. The mapped splicing cis-elements include an exonic splicing silencer (ESS) at nt 612-639 and an exonic splicing enhancer (ESE) at nt 3520-3550 in the virus genome. The ESS suppresses alternative RNA splicing of the HPV18 early polycistronic pre-mRNA from a nt 233 5' splice site to a nt 416 3' splice site, and the ESE promotes alternative RNA splicing of the same polycistronic RNA from a nt 929 5' splice site to a nt 3434 3' splice site. RNA binding assays in search for responsible splicing factors revealed that the identified ESS binds hnRNP A1, whereas the ESE specifically binds SRSF3, the smallest member of the SR protein family. In HPV18 minigene-transfected HEK293 cells, HPV18-infected human primary keratinocytes, and HPV18-positive human cervical cancer cells, knockdown of hnRNP A1 expression leads to promote the nt 233^416 RNA splicing and prevents viral E6 oncoprotein production. In contrast, knockdown of SRSF3 expression inhibits the nt 929^3434 RNA splicing and decreases the expression of E1^E4 viral protein. Both hnRNP A1 and SRSF3 are predominantly expressed in proliferating cervical basal cells, where they keep the expression balance of various HPV18 early genes by regulating alternative RNA splicing of the viral early polycistronic RNA through selective interaction with two viral RNA cis-elements.

P-4

POLY(ADP-RIBOSE) REGULATES microRNA ACTIVITY OF AGO2

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Poly(ADP-ribose) (PAR) is a polynucleotide molecule consisting of 2-200 ADP-ribose subunits as well as a post-translational modification. PAR is covalently attached to its target protein via a family of poly(ADP-ribose) polymerases (PARPs) and modifies its activity/function. In this work, the function of a cytoplasmic RNA-binding PARP "PARP-13" is characterized.

Our laboratory found that overexpression of PARP-13 reduces microRNA (miRNA)-mediated repression, correlating with an increase in PARylation of the core miRNA binding Argonaute (AGO) proteins. Although PARP-13 has PARP domain that is conserved in all PARP family members, the catalytic activity of (ADP-ribosyl)ation is inactive. How PARP-13 modulates AGO2 PARylation remains unclear. Besides the PAR domain, PARP-13 has 5 zinc-finger domains and 1 WWE domain responsible for RNA-binding and PAR-binding, respectively. We found that the RNA-binding of PARP-13 is critical for the reduction of miRNA-mediated repression and one of PARPs could modify AGO2. In this presentation, we will discuss the possible mechanisms on how this inactive PARP PARylates AGO2.

PARP-13 is also known as zinc-finger antiviral protein (ZAP). Besides the full-length protein, cells express an additional alternative splice isoform of PARP-13 that lacks this PARP domain, where such isoform is selectively induced 10- to 40- fold upon viral infection. We found a correlation that miRNA-mediated repression is reduced upon activation of an antiviral response when PARP-13 is upregulated. Moreover, we found PARP-13 could associate with mRNAs of several antiviral response genes upon viral infection. Thus, our research potentially yields new insights into how PARP-13 and PAR regulates post-transcriptional mechanism as a general antiviral response.

P-5

MUTATIONS IN INTEGRATOR COMPLEX SUBUNIT INTS5 AND INTS8 LEAD TO ALTERED GENE EXPRESSION AND ABNORMAL BRAIN DEVELOPMENT

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The Integrator complex (INT) is a large multi-protein complex that is essential for the 3' end formation of the U-rich small nuclear RNAs (UsnRNAs) and has recently also been involved in RNA polymerase II (RNAPII) transcriptional initiation and pause release. INT contains at least 14 subunits and associates with the carboxy-terminal domain (CTD) of the RNAPII. Despite recent progress, most of INT subunits remain structurally and functionally uncharacterized while little is known about the detail of its role in snRNA 3' end formation or in RNAPII initiation and pause release.

We have identified mutations in two INT subunits, INTS5 and INTS8 that are associated with severe neurodevelopmental defects including cerebellar hypoplasia (CbH), and periventricular nodular heterotopia (PNH). In IntS8 mutant cells, we detect reduced levels of several functionally essential Integrator subunits while affinity purified IntS8 mutant complexes fail to associate with these same subunits, reflecting a disruption in the physical integrity of the complex. Importantly, we have uncovered a direct physical interaction between INTS5 and INTS8, suggesting a functional link between the two subunits that may underlie the pathological similarities exhibited between patients. While we identified a small but significant increase in UsnRNAs missprocessing, RNA-seq analysis of patient fibroblast transcriptomes revealed mis-splicing events in several genes known to play a role in differentiation. Additionally, we detected the dysregulation of the expression of large number of genes, many of which could have an impact of neuronal development. Expression data in developing human brain structures and in mouse embryos show that INTS8 expression peaks prenatally in the areas of most actively migrating neurons, in agreement with the observed PNH phenotype. Accordingly, morpholino oligonucleotides mediated knock-down of INTS8 in zebrafish results in underdevelopment of the head and brain.

We propose that INT dysfunction, most probably through altered transcriptional regulation or splicing, leads to severely disrupted brain development in humans. It is the first time that INT subunit mutations are found associated with a human disease. They represent an unprecedented opportunity to study the inner mechanics of the Integrator complex, its function and its broader impact on the physiology of the cell and on neurodevelopment.

P-6

RAPID 4D FRET ANALYSIS OF RIBOSWITCH-LIGAND INTERACTIONS: A NEW APPROACH TOWARDS RNA-TARGETED DRUG DISCOVERY

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RNA biology represents a vastly underexplored field for pharmacological intervention owing, at least in part, to the dearth of appropriate assays and targets currently employed. To develop a robust general assay for RNA-focused drug discovery, we recently explored the interplay between multiple factors influencing RNA-ligand interactions (e.g. the concentrations of salt, Mg^{2+}, ligand, and temperature). We developed an efficient, high throughput method to characterize RNA structure and thermodynamic stability as a function of multiplexed solution conditions using Förster resonance energy transfer (FRET). In a single 4D FRET experiment using conventional quantitative PCR instrumentation, 194,000 conditions of $MgCl_2$, ligand, and temperature were analyzed to generate detailed empirical conformational and stability landscapes of the cyclic diguanylate (c-di-GMP) riboswitch. The method allows rapid comparison of RNA structure modulation by cognate and non-cognate ligands. Landscape analysis revealed the region of highest synergy between Mg^{2+} and c-di-GMP for inducing riboswitch conformational change. In the presence of kanamycin B, a positively charged aminoglycoside, the experimental landscapes exhibited idiosyncratic shapes indicative of altered folding and stability. Biophysical analysis confirmed that kanamycin B stabilizes an alternative, non-native conformation of the riboswitch. This conformation inhibits the binding of c-di-GMP and demonstrates that allosteric control of folding, rather than direct competition with cognate effectors, is a viable approach for pharmacologically targeting riboswitches and other structured RNA molecules.

P-7

A G-QUADRUPLEX FORMING DOMAIN IN THE IRES A OF THE hVEGF FOLDS INDEPENDENTLY AND DIRECTLY RECRUITS 40S RIBOSOMAL SUBUNIT

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RNA G-quadruplexes are secondary structures known to be involved in several processes such as mRNA transcription, translation, editing, splicing amongst others. The GQ structures when present in an IRES act as essential element in contrast to their generally accepted inhibitory role in translation. We observed a 17 nucleotide independently folding RNA G-quadruplex (GQ) domain within the 294 nucleotides long *human VEGF IRES A* that directly interacts with the 40S ribosomal subunit. Footprinting and structure mapping analyses indicate that the RNA GQ forms independently and also interacts directly with the 40S ribosomal subunit in absence of other protein factors. Moreover filter binding assay in conjunction with enzymatic footprinting clearly established that the GQ forming domain singularly dictates the binding affinity and also the function of the IRES A (Internal Ribosomal Entry Site). The deletion of the GQ domain abrogates the 40S ribosomal subunit binding to the IRES, which impairs the cap-independent translation initiation. The findings provide a unique and defined role of a non-canonical RNA structure in the cap-independent translation initiation by cellular IRESs. The results of this study explain the hitherto unknown mechanistic necessity of the GQ structure in IRES function.

P-8

INVESTIGATION OF *glmS* RIBOZYME: ROLE OF EXOGENOUS SPECIES IN SELF-CLEAVAGE MECHANISM

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The *glmS* ribozyme is a small, self-cleaving RNA found in many gram-positive bacteria whose activity requires the presence of a small molecule coenzyme, glucosamine-6-phosphate (GlcN6P), that is believed to serve as the general acid thus making the RNA a riboswitch-ribozyme.[1-4] This ribozyme, along with other similar small ribozymes including the hepatitis delta virus (HDV), hairpin, hammerhead, Varkud satellite (VS), and the newly discovered twister ribozymes, cleaves through a general acid-general base mechanism wherein the 2'-hydroxyl at the cleavage site attacks the adjacent phosphate, resulting in a 2',3'-cyclic phosphate and a 5'-hydroxyl product.[1] The *glmS* ribozyme controls expression of the *glmS* gene, which encodes glutamine/fructose-6-phosphate aminotransferase, an enzyme that produces GlcN6P.[1] Thus, this ribozyme is involved in a self-regulating mechanism wherein high levels of GlcN6P result in cleavage of the ribozyme and subsequent down-regulation of the GlcN6P-producing enzyme. Structural studies on the *glmS* ribozyme indicate that it exhibits a doubly-pseudoknotted, compact active site core that pre-forms prior to GlcN6P binding.[4,5] Key active site residues aid in the binding of GlcN6P, and one residue in particular, G33 in the *B. anthracis* construct, is well-positioned to act as the general base involved in the proton abstraction from the 2'-hydroxyl at the cleavage site in the cleavage mechanism.[6,7] However, studies have shown that G33 exhibits a pK_a shifted away from neutrality ($\geq$10 compared to the pK_a of 9.2 for free guanine in solution).[8] As demonstrated by recent work resulting from a collaboration between our lab and the Hammes-Schiffer lab at UIUC,[9] this basic-shifted pK_a may be required of the general base, wherein a pK_a that more closely matches the pK_a of the 2'-hydroxyl nucleophile would be better suited to abstract its proton. Theoretical studies and pK_a calculations suggest that G33 may serve as a hydrogen bond acceptor to the 2'-hydroxyl nucleophile until attack on the adjacent phosphate begins, when G33 accepts the proton from the 2'-hydroxyl (an event facilitated by the elevated pK_a of G33).[9] The work discussed here, in addition to investigating the function of G33 in the cleavage mechanism, aims to determine the role of exogenous species, including divalent metal ions, in *glmS* cleavage, and to probe the cleavage mechanism under biological conditions. Previous studies as well as preliminary results suggest that divalent metal ions, particularly Mg^{2+}, greatly enhance the rate and extent of cleavage. Current and future studies include optimization of reaction conditions to achieve monophasic, complete kinetics and further characterization of the role of divalent metal ions, whether it be catalytic or purely structural.

1. Winkler, W. C.; Nahvi, A.; Roth, A.; Collins, J. A.; Breaker, R. R., Nature 2004, 428, 281-286.
2. McCarthy, T. J., Plog, M. A., Floy, S. A., Jansen, J. A., Soukup, J. K., and Soukup, G. A. Chemistry & Biology 2005, 12, 1221-1226.
3. Viladoms, J. and Fedor, M. J. J. Amer. Chem. Soc. 2012, 134, 19043-19049.
4. Klein, D. J. and Ferré-D'Amaré, A. R. Science 2006, 313, 1752-1756.
5. Cochrane, J. C., Lipchock, S. V., Smith, K. D., and Strobel, S. A. Biochemistry 2009, 48, 3239-3246.
6. Cochrane, J. C.; Lipchock, S. V.; Strobel, S. A., Chem.Biol. 2007, 14, 97-105.
7. Klein, D. J.; Been, M. D.; Ferré-D'Amaré, A. R., J. Amer. Chem. Soc. 2007, 129, 14858-14859.
8. Viladoms, J.; Scott, L. G.; Fedor, M. J., J. Amer. Chem. Soc. 2011, 133, 18388-18396.
9. Zhang, S., Ganguly, A., Goyal, P., Bingaman, J. L., Bevilacqua, P. C., and Hammes, Schiffer, S. J. Am. Chem. Soc. 2015, 137, 784-798.

P-9

POST-TRANSCRIPTIONAL COORDINATION OF GENE EXPRESSION BY HUR AND microRNAs DURING BREAST CANCER PROGRESSION

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Aberrant gene expression is an important factor in tumorigenesis, yet little is known about the role RNA-binding proteins (RBPs) play in disease onset and progression. Sequence specific RBPs coordinately regulate subsets of functionally related mRNAs within ribonucleoprotein complexes (RNPs), which are remodeled in response to cellular perturbations, allowing for the rapid and coordinated expression of proteins that have common functions. These post-transcriptional events robustly influence expression patterns of proto-oncogenes, growth factors, cytokines, and cell cycle regulators by influencing both mRNA stability and translation. Here we investigate mechanisms of RNA regulation central to breast cancer progression. We have identified transcriptomic changes during the stepwise transition from primary mammary epithelial cells to a fully malignant state, as well as coordinated RNA dynamics of transient cellular RNPs. For this analysis, we generated an isogenic model of human breast cancer formation in which normal mammary epithelial cells were first immortalized through the expression of hTERT, $p53^{DD}$, Cyclin D1, $CDK4^{R24C}$ and $C\text{-}MYC^{T58A}$, and then subsequently transformed by the addition of $H\text{-}RAS^{G12V}$. In this system, the most significant transcriptomic changes occur during early tumorigenesis. Genes most significantly changed are those involved in cell adhesion and cadherin signaling. Consequently, we show that immortalized cells have acquired migratory capabilities. Surprisingly, although no significant transcriptomic changes were observed during RAS transformation, fully transformed cells have acquired invasive capabilities. While mRNA encoding N-cadherin (CDH2), a prototypical mesenchymal protein, is robustly expressed in both immortalized and transformed cells, N-cadherin protein is only detected in the fully malignant state. Accordingly, CDH2, as well as several other functionally related mRNAs, exhibits an increased half-life and polysome association in transformed cells. Therefore, our data suggest RAS-dependent post-transcriptional regulation that contributes to metastatic potential. To investigate the role of RBPs, we used RNP-immunoprecipitation followed by high-throughput sequencing to quantify the remodeling of mRNA subsets associated with the RBP HuR, a translational activator known to target many cancer-related mRNAs. Here we present evidence that HuR regulates subsets of mRNAs in a dynamic manner. Association dynamics are observed for CDH2 and other cell adhesion-related mRNAs, suggesting that HuR may contribute to enhanced mRNA stability and translation of subsets of mRNAs that enhance metastatic potential during RAS transformation. In addition, we show that RAS transformation enhances suppression of a subset of microRNAs that acts as inhibitors of invasion and expression of a subset of microRNAs that activates invasion. HuR has been demonstrated to compete with microRNAs for the fate of common mRNA targets. Our data suggest a complex and dynamic regulation by microRNAs and RBPs during RAS transformation, and we are currently working to better understand these dynamic interactions and their precise role in tumorigenesis on a global scale. The results from this study may ultimately direct our ability to counter the RNA regulatory changes that underlie malignancy.

P-10

NEUTROPHIL EXTRACELLULAR TRAPS AS CARRIERS OF RNA

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Extracellular traps are extruded by neutrophils, among other innate immune cells, in response to pathogens and certain inflammatory stimuli. Neutrophil derived extracellular traps (NETs) can immobilize pathogens, thereby contributing to their destruction. NETs contain extruded nuclear components decorated with selected granular enzymes. Recent evidence implicates abnormal NET formation in the development of various systemic autoimmune disorders including systemic lupus erythematosus (SLE). Indeed, an abnormal neutrophil subset present in SLE patients, termed low-density granulocytes (LDGs) display significantly enhanced NET formation in the absence of infection. While DNA and chromatin have been reported in the NETs, it is unclear if other nuclear components become extruded during this process. Further, the immunomodulatory role that various nuclear components present in the NETs play remains unclear. RNA and, particularly, small RNAs are increasingly described for their central roles in signaling between cells. We hypothesized that NETs serve as carriers of immunomodulatory RNAs that may regulate important cellular responses. In the present work we sought to determine first if RNA was present in the NETs. Next, we characterized what types of RNAs may be differentially expressed in the NETs derived from lupus patients versus healthy controls. Finally, we investigated if RNA externalized in NETs could be internalized into innate immune cells, thereby modifying their responses. Ongoing work is attempting to further elucidate an additional role of NETs as carriers of RNA and, potentially, broad regulators of cellular responses.

P-11

ALLELE-SPECIFIC SILENCING OF A DOMINANT-NEGATIVE MUTATION IN ULLRICH CONGENITAL MUSCULAR DYSTROPHY CELLULAR MODELS USING siRNA OR LNA ANTISENSE OLIGONUCLEOTIDES

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Congenital muscular dystrophy type Ullrich (UCMD) is a severe progressive disorder of early childhood onset, presenting with generalized muscle weakness, distal joint hypermobility, proximal joint contractures and respiratory failure as the main features. At present, there are no pharmacological treatment options available for children affected with this disease. In the laboratory we aim at exploring targeted RNAi and antisense approaches as potential therapies for UCMD. Dominant and recessive mutations in the three genes coding for collagen type VI (*COL6A1*, *COL6A2*, *COL6A3*) underlie UCMD, with dominant-negative mutations accounting for the majority of cases. Achieving allele-specific silencing of the mutant collagen VI transcript would convert this dominant-negative state into a clinically asymptomatic haploinsufficient state. We have previously demonstrated the allele-specificity and efficiency of siRNA oligos to downregulate the expression of a mutant *COL6A3* transcript *in vitro*. We have now extended our study to Locked Nucleic Acid (LNA)-modified oligonucleotides, which are short, stable, and RNaseH-recruiting oligonucleotides that can be delivered to the cell without any carrier. We used outcome measures such as unsaturated PCR, quantitative RT-PCR and immunoblot on treated cell lysates, and confocal microscopy on treated fixed cells, and found that both RNAi and RNase-H pathways were comparably effective. This study provides further insights into the comparative allele-specificity of these two pathways to target dominant mutations at the transcript level, with the goal of developing optimal compounds for *in vivo* application.

P-12

STRUCTURAL INSIGHTS INTO THE STABILIZATION OF MALAT1 NON-CODING RNA BY FORMATION OF A BIPARTITE TRIPLE HELIX

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Human metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is an abundant, ~8-kb long non-coding RNA (lncRNA) that is upregulated in multiple cancers and promotes malignancy. Interestingly, MALAT1 terminates with a short, genomically-encoded A-rich tract, rather than a canonical 3′-poly(A) tail, yet decays with a half-life up to 15 h. We hypothesized that MALAT1's abundance is due to the stabilizing formation of a 3′-triple-helical structure in which the A-rich tract is engaged with an upstream U-rich internal loop of a predicted ENE (expression and nuclear retention element), a stem-loop structure similar to those found in viral lncRNAs and genomic RNAs.

We have discovered that the ENE region is critical for the accumulation of full-length MALAT1 in cells. Furthermore, MALAT1 sequences containing the ENE and A-rich tract (ENE+A) increase the levels of an intronless β-globin reporter RNA. The MALAT1 ENE+A structure forms a triple helix based on assay results from mutant β-globin reporters, UV thermal denaturation profiles at different pH values and a 3.1-Å resolution crystal structure of the MALAT1 ENE and downstream A-rich tract.

The crystal structure shows that the MALAT1 ENE+A forms a bipartite triple helix composed of nine U•A-U triples, a single C•G-C triple that is likely protonated *in vivo*, and a C-G doublet. The C-G doublet divides the triplex into one stack of four U•A-U triples and one stack of five U•A-U triples capped by a C^+•G-C triple. The unique C^+•G-C triplet/C-G doublet serves at least three major functions: (i) it aligns the A-rich tract and U-rich loop to position the 3′-terminal A in a U•A-U triple, creating a blunt-ended triplex; (ii) it stabilizes a mostly U•A-U RNA triple helix by increasing base-stacking interactions; and (iii) the C-G doublet functions as a "helical reset" mechanism to preserve base triple interactions for two independent stacks of triples, providing a structural rationale for why triple-helical stacks longer than six have not been found in nature. The bipartite triple-helical structure is extended by two A-minor interactions with G-C base pairs.

In vivo decay assays indicate that this blunt-ended, triple-helical structure inhibits the rapid phase of RNA decay. To maintain robust stabilization activity, the MALAT1 ENE+A structure requires an intact triple helix, strong stems at the duplex-triplex junctions, a G-C base pair flanking the triplex to mediate A-minor interactions, and a blunt-ended triplex lacking unpaired nucleotides at the duplex-triplex junction. Finally, our β-globin reporter assays support this ENE-based mechanism to counteract decay for another abundant cellular lncRNA: multiple endocrine neoplasia beta (MENβ).

P-13

NOVEL PIWI/AGO FAMILIES WITH ROLES IN MEDIATOR-DEPENDENT TRANSCRIPTION IN EUKARYOTES AND BACTERIAL INTER-GENOME CONFLICT THROW LIGHT ON THE EARLY EVOLUTION OF RNAi SYSTEMS

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The PIWI module, found in the PIWI/AGO superfamily of proteins, is a critical component of several cellular pathways including germline maintenance, chromatin organization, regulation of splicing, RNA interference, and virus suppression. It binds a guide strand which targets complementary nucleic strands as part of these pathways. We recently discovered two divergent, novel families of PIWI modules, the first such to be described since the initial discovery of the PIWI/AGO superfamily over a decade ago. Both families conserve the amino acid residues necessary for the binding of oligonucleotide guide strands. The first family, found only in eukaryotes and named the MedPIWI family, is the core conserved module of the Med13 protein, a subunit of the CDK8 subcomplex of the transcription regulatory Mediator complex. Med13 functions as part of a regulatory switch through which the CDK8 subcomplex modulates transcription at Mediator-bound promoters of highly transcribed genes and we propose this switch is activated via recognition of small RNAs by the MedPIWI module, resulting in a conformational change that propagates throughout the Mediator complex. The second family, bacterial in distribution and named the PIWI_RE family, is one part of a highly-mobile, three-gene operon which also includes a restriction endonuclease fold enzyme and a helicase of the DinG family. The presence of the DinG family helicase, which specifically acts on DNA-RNA hybrid-forming R-loops, implicates PIWI_RE as a sensor in a novel RNA-dependent restriction system potentially targeting invasive DNA from phages, plasmids or conjugative transposons.

Placement of these novel families in the broader context of PIWI/AGO evolutionary history adds to increasing evidence that ancestral members of the superfamily functioned in bacterial conflict systems targeting DNA substrates. The shift to RNA-targeting occurred later in the diversification of the family, quite possibly prior to the recruitment of PIWI/AGO to RNAi systems during eukaryogenesis. The MedPIWI family reveals that at least two versions of the PIWI/AGO module were recruited to distinct functional contexts in the early phases of eukaryotic evolution.

P-14

ANTISENSE OLIGONUCLEOTIDE-MEDIATED DMPK REDUCTION DOES NOT AFFECT CARDIAC CONDUCTION OR EJECTION FRACTION IN MICE

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Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy of adults. Cardiac involvement ranks second as a cause of death in DM1, due to heart block or tachyarrhythmia. DM1 is a dominant disorder caused by an expanded $(CTG)_n$ repeat in the 3`-untranslated region of the *DM Protein Kinase (DMPK)* gene. Transcription of the mutant allele leads to production of CUG-expanded *DMPK* transcripts in muscle and heart. The mutant transcripts are retained in nuclear foci and DMPK protein levels are reduced by half. The cardiac conduction system defects in DM1 have been attributed to DMPK reduction, toxicity of CUG-repeat RNA, or both. Previous work has shown that mice lacking DMPK develop PR interval prolongation and atrioventricular block. Antisense oligonucleotide (ASO) drugs targeting toxic (CUG)-containing transcripts are capable of producing strong knockdown and reversal of disease phenotypes in skeletal muscle of transgenic mice. Clinical trials of ASO drugs for DM1 were recently initiated. While the ASO drugs have potential to treat RNA toxicity in the heart, there is also risk of aggravating the DMPK deficiency.

To better understand the potential effects of DMPK-targeting ASOs on cardiac function, we studied mice with congenital or acquired DMPK reduction: homozygous *Dmpk* knockout (-/-) mice, or heterozygous *Dmpk* knockout (+/-) mice treated with saline or *Dmpk*-targeting ASOs. Beginning at age 2 months, the ASO was administered to +/- mice by weekly subcutaneous injection at 50mg/kg for 6 weeks, then biweekly. As compared to wild-type (WT) controls, *Dmpk* mRNA was reduced by 84 ± 3% in the hearts of ASO-treated +/- mice, and protein was reduced by 93 ± 2%. ECG measurements at 6 months demonstrated no difference of heart rate or cardiac conduction in WT, +/- saline, +/- ASO, or -/- mice, and echocardiography showed no difference of ejection fraction (78% in WT, 75% in +/- saline, 73% in +/- ASO and 76% in -/-, ANOVA p=0.22). ECG measurements and echocardiograms at 10 months again showed no difference of heart rate or cardiac conduction, and ejection fraction remained normal (73% in WT, 76% in +/- saline, 75% in +/- ASO and 71% in -/- , ANOVA p=0.45). Conscious, unrestrained ECGs were obtained at 11-12 months of age using radio telemetry and signal averaging. Once again, heart rate and cardiac conduction measurements showed no difference in WT, +/- saline, +/- ASO, and -/- mice. These results demonstrate the potential of ASOs to induce silencing of *Dmpk* expression in the heart, and indicate that acquired Dmpk reduction that is well below heterozygous levels (7% of WT) is well tolerated. Taken together with other findings, our data support the concept that cardiac dysfunction in DM1 predominantly results from RNA toxicity, which potentially is mitigated by treatment with ASOs.

P-15

PROFILING OSTEOGENIC microRNAs FOR RNAi-FUNCTIONALIZATION OF SCAFFOLDS IN BONE TISSUE ENGINEERING

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Bone remodeling and bone repair are natural processes occurring in the body throughout life. This metabolic process ensures that microfractures and microinjuries are repaired and thus maintaining healthy bones. However, when the damage is too large, such as from trauma injury or bone tumors, repair is limited and grafts are required to assist in bone repair. The use of allografts can cause immunological complications, whilst autografts subject the patient to two surgeries. Bone tissue engineering is a multidisciplinary field encompassing material science, medicine, chemistry and molecular biology aimed to produce a functional graft *in vitro* as an alternative to allografts and autografts. We explored the microRNAs (miRNAs) that aid in the bone formation process. MiRNAs are small non-coding RNAs of about 17-22 nucleotides in length that target the 3'UTR of mRNAs and represses their expression. MiRNAs have been found to facilitate many processes in the body including development, metabolism, and are implicated in many diseases. Many miRNAs have been identified with roles in osteogenesis, however a large systematic view at miRNA expression throughout osteogenesis that includes early, intermediate and late time points has yet to be done. We aimed to identify the expression profiles of miRNAs that as mesenchymal stem cells underwent osteogenesis with microRNA-sequencing. Most miRNAs were downregulated during osteogenesis and only few were upregulated. With the use of weighted gene correlated gene analysis we identified several expression profiles. Several miRNAs were validated in their osteogenic capabilities by overexpressing and knocking down the miRNAs, then assessing their ALP activities, Alizarin Red and ALP stainings and the expression of osteoblastic markers. In our screen we have identified both miRNAs that have been reported previously and many novel miRNAs with potent osteogenic capabilities. For tissue engineering applications, we then functionalized scaffolds with the miRNAs we identified and observed an increase in osteogenic capabilities in our 3D cultures. Our findings depicted the miRNA expression landscape as mesenchymal stem cells underwent osteogenic differentiation. We also highlight the potency of miRNAs as biological therapeutics in bone tissue engineering.

P-16

A METHOD TO EFFICIENTLY SELECT mRNA TARGETS AND IDENTIFY sRNA REGULATORS

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Small regulatory RNAs are present in all three kingdoms of life, and their essential roles in developmental biology and cellular physiology have been well documented. Bacterial small regulatory RNAs (sRNAs) are key stress-response regulators that can shield bacteria against adverse conditions through fine-tuning a broad repertoire of stress-response genes. Mechanistically, bacterial sRNA regulation is achieved through controlling the stability and/or translation of target mRNAs by an antisense mechanism. The global sRNA chaperone Hfq plays critical roles in this post-transcriptional regulation, and thus far ~30 Hfq-dependent sRNAs regulating ~100 mRNA targets have been found in *Escherichia coli*. However, an efficient and reliable method to select candidate mRNA targets of interest is not readily available yet.

In this study, we present an effective and efficient combinatorial method to select mRNA targets and identify sRNA regulators. The candidate mRNA targets were selected based on physical association with Hfq protein plus transcript level change in the presence versus absence of Hfq. By analyzing genomic data (RNA microarray and RIP tiling-array), we predicted ~100 candidate mRNA targets that highly associate with Hfq and largely change transcript levels in the *hfq* mutant. We further validated the selection method by experimentally identifying sRNA regulators for 3 selected candidate mRNA targets (*recA*, *mutS* and *yhcN*) using a reporter-based, sRNA overexpression library method (Mandin & Gottesman, 2010). We report here that Spot42 sRNA strongly represses *recA* and *yhcN*, and ArcZ sRNA represses *mutS* gene expression, both by a post-transcriptional mechanism. More importantly, we showed that in all cases ablation of physiological levels of the sRNA is sufficient to disturb target mRNA expression, indicating a bona fide regulation related to the bacterial physiology. Taken together, this study builds a pipeline for efficiently identifying sRNA regulators involved in specific biological events of interest by providing guidelines for selecting candidate mRNA targets and experimentally identifying and validating sRNA regulators in action.

P-17

THE AUTOPHAGY RECEPTORS P62 AND NDP52 SELECTIVELY TARGET STRESS GRANULES AND P-BODIES FOR DEGRADATION

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Autophagy is a conserved process through which cytoplasmic components are sequestered in a double-membraned vesicle and subsequently routed to the lysosome for degradation. The process is constitutively active and is upregulated in response to environmental stresses. Interestingly, autophagy does not randomly degrade cytoplasmic content; autophagy receptors such as p62, NDP52 and NBR1 link specific cargoes to the nascent autophagosome membrane for degradation. Autophagy, an essential homeostatic mechanism, is implicated in the pathogenesis of cancer, cardiomyopathy, and several neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). Pathology is often mediated by accumulation of toxic autophagic substrates, but these have been identified only in rare circumstances. The field of autophagy has focused on proteins and organelles as the substrates for degradation, despite evidence that a major fraction of RNA is degraded by autophagy, particularly in stress. We hypothesized that RNA-rich stress granules (SGs) and processing bodies (PBs) are selectively targeted for degradation by autophagy receptors.

We found that p62 and NDP52 are preferentially recruited to stress granules and P-bodies respectively, as detected by expressing GFP-Dcp1a and mCherry -TIA-1. Further NDP52 preferentially co-localized with endogenous markers of P-bodies rather than stress granules, while p62 co-localized with endogenous stress granules induced by oxidative stress. A third autophagy receptor, NBR1, co-localized with a subset of these stress granules and P-bodies. Several components of stress granules, including DDX3, immuno-precipitated with p62. P-bodies and stress granules frequently co-localized with LC3 - a marker of autophagosomes and depletion of NDP52 and p62 caused the number of P-bodies and stress granules per cell to increase respectively. Overall, this strongly suggests that P-bodies and stress granules are preferentially recognized by distinct autophagy receptors and recruited to forming autophagosomes for selective degradation. Autophagy may be an effective mechanism to regulate bulk turnover of RNA regrouped in granules. Intriguingly, emerging evidence suggests that pathology in ALS involves inefficient autophagic clearance of mutant proteins in stress granules. Our identification of p62 as a selective autophagy receptor for degradation of stress granules, suggests a mechanism for the genetic implication of p62 in ALS.

H. Guo, M. Chitiprolu, D. Gagnon, L. Meng, C. Perez-Iratxeta, D. Lagace, and D. Gibbings, 'Autophagy Supports Genomic Stability by Degrading Retrotransposon RNA', *Nature Communications*, 5 (2014), 5276.

P-18

MESH ON DEMAND: AN EASY WAY TO IDENTIFY RELEVANT MESH TERMS AND RELATED ARTICLES FROM TEXT

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The National Library of Medicine (NLM) has developed MeSH on Demand, a Web-based tool that recommends Medical Subject Heading (MeSH®) terms from text such as an abstract or grant summary using the NLM Medical Text Indexer (MTI) software. MeSH on Demand has been developed in close collaboration between MeSH, NLM Index Section, and the Lister Hill National Center for Biomedical Communications to address this need.

The NLM Medical Text Indexer (MTI) is the main product of the Indexing Initiative project and has been providing indexing recommendations based on the MeSH vocabulary since 2002. MTI combines human NLM Index Section expertise and Natural Language Processing technology to curate the biomedical literature more efficiently and consistently. In 2011, NLM expanded MTI's role by designating it as the first-line indexer (MTIFL) for a select number of journals where it performs particularly well; today the MTIFL workflow includes over 200 journals for which MTIFL provides initial indexing that is then curated by human indexers.

The MeSH on Demand tool is now freely available at http://www.nlm.nih.gov/mesh/MeSHonDemand.html and requires no software downloads or prior knowledge of the MeSH vocabulary. Users simply input text in the box labelled "Text to be processed." The input text can contain up to 10,000 characters and may include a variety of text types including title, abstract, grant summary, or keywords. Well-defined sentences provide the best results. The user then selects the "Find Mesh Terms" button, and MeSH on Demand provides as a result a list of MeSH terms that MTI identifies as being relevant to the text; the terms are displayed in alphabetical order, and a link to the MeSH Browser is provided for each term. For example, with input text that contains the phrase "treatment-resistant depression," MeSH on Demand automatically identifies the MeSH heading *Depressive Disorder, Treatment-Resistant*. MeSH on Demand also includes links to the top ten related citations in PubMed (in ranked order) that were used in computing the MeSH on Demand results.

Although the MeSH terms provided by MeSH on Demand are machine generated by the MTI software and do not reflect any human review, they do provide a good summary of the text.

P-19

DISTINCT PATHWAYS FOR LOCALIZATION OF RNAs AT CELL PROTRUSIONS

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Recent data suggest that localization of RNAs to specific subcellular compartments is more the rule than the exception, but to date the mechanisms and factors involved remain largely uncharacterized. Our lab has identified, through a genome-wide screen, hundreds of RNAs enriched in protrusions of migrating fibroblast cells. We observed that some of these mRNAs rely on microtubules and the tumor-suppressor protein APC for their localization. We have now determined that a distinct subset of protrusion-enriched RNAs relies on a different localization pathway. Interestingly this subset comprises virtually all of the approximately 80 ribosomal protein mRNAs. Unlike other mRNAs enriched in fibroblast protrusions, localization of r-protein mRNAs does not appear to depend upon the microtubule network or the tumor suppressor APC. Furthermore, r-protein mRNAs and APC-dependent RNAs exhibit opposing preferences for enrichment towards distinct types of protrusions. APC-dependent RNAs show higher enrichment in microtubule-containing protrusions, while r-protein mRNAs become mostly enriched in actin-rich protrusions. Thus, r-protein mRNAs appear to represent a new, differentially regulated class of localized mRNAs. We are identifying the mechanisms and additional components of this localization pathway.

P-20

INHIBITION OF HIV-1 AND ADENOVIRUS REPLICATION USING A MODULATOR OF RNA PROCESSING

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Replication of numerous mammalian viruses is critically dependent upon the balanced splicing and processing of their RNAs. To explore the therapeutic potential of targeting viral RNA processing, we have examined the capacity of multiple small molecule modulators of RNA splicing for their ability to suppress replication of both HIV-1 and adenovirus, two very distinct viruses that share a requirement for tight regulation of viral RNA metabolism. In a screen of compounds that alter RNA splicing for antiviral activity, we identified a benzoxadiazol-4-amine derivative (191) that markedly suppressed structural protein expression in the context of both HIV-1 and adenovirus. In the case of HIV-1, treatment of chronically infected cells with 191 resulted in a marked reduction in the levels of viral RNAs encoding structural proteins (Gag and Env) but had no effect on the abundance of viral RNAs encoding Tat or Rev. However, 191 treatment blocked Tat and Rev protein accumulation, indicating that it is functioning at several levels to repress HIV-1 gene expression. Similar anti-HIV-1 activity was seen in the context of multiple cell lines (HeLa, SupT1, CEM-T4) and preliminary data indicates that 191 inhibits HIV-1 replication in PBMCs. In parallel, studies, treatment of cells with 191 following infection with adenovirus resulted in a 2-3 log reduction in virus yield. Treatment with 191 had little or no effect on adenoviral early protein (E1A) expression but dramatically decreased late protein (hexon) synthesis. Subsequent analysis of viral RNA revealed that 191 altered the splicing of early adenoviral transcripts and blocked the accumulation of mRNAs for the late genes, consistent with an inhibition of the transition from early to late adenoviral gene expression. Effects on viral replication were observed at concentrations of 191 that had little or no effect on cell viability and altered only 2.3% of host mRNA exon inclusion events by more than 20%. Consequently, these studies have demonstrated that a modulator of RNA processing can effectively suppress replication of distinct viruses, suggesting that this approach could prove useful for the treatment of infections that either lack therapeutics (adenovirus) or where resistance remains problematic (HIV-1).

P-21

INTRON RETENTION IS A MAJOR REGULATOR OF GENE EXPRESSION DURING TERMINAL ERYTHROPOIESIS

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We previously showed that differentiating erythroblasts execute a dynamic alternative splicing program involving many cassette exons. Here we used new computational tools to elucidate a novel network of intron retention (IR) events that play an important role in modulating the transcriptome during terminal erythropoiesis. Differences in differentiation stage-specificity, degree of retention, nuclear/cytoplasmic localization, and sensitivity to nonsense-mediated decay (NMD) suggest the existence of multiple IR classes subject to distinct regulatory controls. We analyzed a set of IR events comprised of highly-retained single introns and pairs of introns in otherwise efficiently-spliced transcripts. These stably-expressed IR transcripts were primarily nuclear-localized, where they could avoid NMD and potentially undergo final processing in response to physiological stimuli. Gene ontology (GO) analysis revealed that IR was enriched in major genes for RNA processing, likely influencing expression of numerous downstream splicing targets. Genes with critical functions in iron homeostasis were also affected by IR. High IR was enriched adjacent to alternative NMD-inducing exons, suggesting a mechanistic association with unproductive splicing events; in contrast, retention was lower for introns flanking alternative coding exons and constitutive exons. High IR was also observed in important disease genes including splicing factor SF3B1 (myelodysplasia), transferrin receptor-2 (hemochromatosis type 3); and the RNA binding protein FUS (ALS). We propose that IR plays a critical role in gene regulation during normal erythropoiesis, and mis-regulation of IR may be responsible for human disease.

P-22

ONCOGENIC ACTIVATION OF THE RNA BINDING PROTEIN NELFE IS MEDIATED BY C-MYC SIGNALING IN HEPATOCELLULAR CARCINOMA

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Global transcriptomic alterations of both coding and non-coding RNA species are a ubiquitous feature associated with cancer. Deregulation of RNA-binding proteins (RBPs) contributes to the transcriptome imbalance in cancer as they regulate RNA processing by modulating the maturation, stability, transport, editing and translation of RNA transcripts through their modular domains. We hypothesize that the activation of RBPs is a novel mechanism that contributes to global aberrant transcriptomic changes in hepatocellular carcinoma (HCC). We analyzed genomic alterations amongst a family of 860 RBPs that was recently identified from *in-vivo* ultraviolet light-induced cross-linking of proteins to RNA using the Liver Cancer Institute (LCI) cohort of 241 HCC and matched non-tumor samples and the COSMIC database (1730 HCC cases). We found that more than 70% of RBPs are dysregulated at the transcriptome level, are more likely to be activated in tumors, and are associated with HCC prognosis. Moreover, RBPs are more likely to acquire somatic mutations than non-RBPs. These mutations had a higher frequency of somatic mutations within the RNA binding domains (RBDs) and low complexity sequences (LCS). To investigate the functional role of disease-associated mutations that are frequently found in the LCS or RBDs, we identified Negative Elongation Factor E (NELFE), as an RBP candidate for functional studies. While the activation of NELFE induces an oncogenic phenotype, the abrogation of NELFE in HCC cells significantly decreased cancer associated phenotypes such as cell proliferation, migration/invasion and tumorigenecity *in vivo*. We identified more than 1500 NELFE dependent genes, of which 494 were tumor-specific NELFE dependent genes using transcriptomic analyses. GSEA analysis of tumor-specific NELFE dependent genes shows that there is enrichment for activated c-Myc targets in HCC. Interestingly, NELFE c-Myc target genes were able to separate tumor from non-tumor in two independent data set, suggesting that NELFE c-Myc targets are tumor specific. Moreover, NELFE c-Myc target genes are associated with survival in two independent cohorts, whereas NELF-E dependent non-MYC target genes are not associated with survival. Further investigation of whether disease-associated mutations that are frequently found in the LCS or RBDs are functionally important demonstrates that the loss of the LCS domain, but not the RBD of NELFE, has a significant effect on cell proliferation. Together our data suggest that the activation of NELFE selectively activates c-Myc target genes in HCC, possibly through the LCS domain. Currently, we are investigating how c-Myc target genes are regulated by NELFE in HCC.

P-23

HIGH-THROUGHPUT SINGLE-MOLECULE SCREEN FOR SMALL-MOLECULE REGULATORS OF SPLICING

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The synthesis of mRNA is a complex and highly regulated process. In eukaryotes, mRNA synthesis is catalyzed by RNA polymerase II and involves several distinct steps such as transcript initiation, elongation, splicing of introns, cleavage, and transcript release. Certain small molecules, such as inhibitors of topoisomerase I, can alter the kinetics of transcription, specifically transcript elongation. To determine specific enzymes that affect transcription kinetics we have developed a high-throughput fluorescence microscopy approach to screen a wide array of small molecules. By fluorescently labeling the intron and exon of a reporter gene, we are able to measure the number of transcripts at the transcription site and extract approximate elongation, splicing and release rates from these measurements. We then validated the results from the high-throughput screen by a live-cell single-molecule imaging assay. We found that 5 of the 25 compounds screened affected elongation rates, 3 of those targeted BRD4 a member of the BET family of proteins, one targeting HDAC and one SIRT activator. These results are suggest a model that BRD4 regulates transcript elongation by removal of hyperacetylated nucleosomes.

P-24

DIFFERENTIAL mRNA AND microRNA EXPRESSION IS INFLUENCED BY RACE IN HYPERTENSIVE AND NON-HYPERTENSIVE WOMEN

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Systemic arterial hypertension occurs more frequently among African Americans (AA) than any other population sub-group, has earlier onset, and more frequently results in end-organ complications. In addition, AA women have the highest incidence and hospitalization rates for hypertension. Previous data suggests that gene expression patterns may increase individual susceptibility to selected chronic diseases. Therefore, we hypothesized that differential gene expression may influence the disproportionate incidence and prevalence of hypertension among AAs. Transcriptional profiling of peripheral blood mononuclear cells (PBMCs) from AA or White, normotensive or hypertensive females identified thousands of mRNAs differentially expressed by race or presence of hypertension. Since microRNAs (miRNAs) are well-known post-transcriptional regulators of mRNA expression levels, we additionally profiled global miRNA expression in the same cohort. Analysis of microarray expression changes using Ingenuity Pathway Analysis identified mRNA-miRNA regulatory networks in hypertension-related pathways, i.e. the renin-angiotensin system, nitric oxide signaling, and actin cytoskeletal signaling pathways, which differ by race and hypertension status. mRNA and miRNA gene expression changes were validated using RT-qPCR in an expanded cohort and gene functionality was investigated *in vitro* using human umbilical vein cells. We have identified several miRNAs, including miRs 20a-5p, 30c-5p, 4763-5p, and 4717-3p, which are significantly and differentially expressed by race and/or presence of hypertension. Together, these findings identify several pathways and miRNA gene candidates whose expression differ with race and may contribute to hypertension-related health disparities and could potentially serve as biomarkers for hypertension or targets for therapeutic modalities.

P-25

A ROLE FOR EPS-URINE-DERIVED MIRNAS AS DIAGNOSTIC AND THERAPEUTIC TARGETS FOR PROSTATE CANCER

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Prostate cancer remains the second leading cause of cancer-related deaths among men in the United States despite the widespread use of prostate specific antigen (PSA) as a diagnostic marker for prostate cancer and as a predictor of treatment outcome. MiRNAs have emerged as crucial regulators of cancer progression with immense diagnostic and therapeutic potential. MiRNAs are often dysregulated in human prostate cancers. However, it is poorly understood how these small RNAs function in the prostate to promote cancer progression. Our laboratory profiled human metastatic primary prostate tissue and cell lines in order to identify novel prostate-cancer associated miRNAs. We screened these candidates in an innovative prostatic fluid biomarker source called expressed prostatic secretions in urine (EPS urine) to determine their utility as discriminating biomarkers for advanced forms of prostate cancer. EPS is collected non-invasively via urine capture following gentle massage of the prostate during a routine digital rectal exam, causing the release of prostatic fluids and detached epithelial cells directly into the urethra. 10 out of 50 miRNA candidates tested showed statistically significant differences in expression between high- and lower-grade cancer patients and non-cancer patient groups within EPS urine. Increased expression of one of these, miR-888, correlated with advanced prostate cancer in these fluids. Functional assays revealed that miR-888 promotes proliferation, migration, and colony formation of hormone-refractory and androgen-sensitive human prostate cancer cells. Our working model is that miR-888 overexpression in the diseased prostate suppresses SMAD4, RBL1, and TIMP2 targets leading to tumor progression and metastasis. Human miR-888 belongs to a genomic cluster of seven miRNAs on human chromosome Xq27.3. We are currently testing how this miR-888 cluster regulates prostate progression using animal models and how miRNA-loaded exosomes modulate tumorigenesis in mice. This research will provide a better understanding how non-coding RNAs influence prostate tumor cells progress to a more aggressive state.

P-26

BRIDGING THE GAP BETWEEN RNA BASED PROTOCELLS AND MULTIPLE TURNOVER IN RNA RIBOZYMES

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It is widely accepted that RNA once acted as both the heredity material and cellular machinery of early life.[1] While this hypothesis offers many attractive features, there are still many challenges protocells would have had to overcome without being afforded the luxury of modern cellular machinery.[2] One important function for the proliferation of life using a polymeric genetic storage material like nucleic acids involves the replication of that material.[3] This becomes problematic because strand dissociation and exchange becomes rate limiting. Energy must be added to separate strands apart as complementary pairing of nucleic acids is a spontaneous process under cellular conditions. In contemporary cells, protein helicases separate double-stranded nucleic acids,[4] but these would not have been available in an early life scenario. Another important function includes concentrating otherwise dilute nucleic acids into a small enough volume where catalysis and reactions can occur. In areas such as thermal vents and rock surfaces, the effective volume can be vast, causing nucleic acid concentrations to be very low.[5] With so many scenarios plausible for the emergence of life, addressing the two challenges of strand separation and co-localization of genetic material must be explored.

Herein we report that alkaline conditions drive product release during a two-piece hammerhead ribozyme reaction under both single-turnover and multiple-turnover conditions. This ribozyme is ideal for studying effects of pH and compartmentalization on catalysis because its kinetics have been well-characterized under a variety of conditions[6, 7, 8] and its sequence can be lengthened in a simple and rational manner. In a related approach, we also mimicked intracellular compartmentalization and crowding by producing complex coacervates composed of anionic RNA nucleotides with cationic poly(allylamine). We describe the characterization of complex coacervates composed of nucleotides (adenosine tri-, di- or monophosphate) and poly(allylamine) under varying pH and ionic strength, as well as the partitioning properties of RNA and magnesium into these systems. Excellent partitioning of RNA into the coacervate was observed under all conditions, while changes in magnesium ion partitioning and surface charge were seen as a function of ionic strength and pH, leading to a better understanding of environments where coacervation of nucleic acid material would have occurred. In the future, the combination of these two approaches will allow for the production of a functional protocell able to undergo strand exchange and catalysis under multiple turnover conditions.

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1. Atkins, J. F.; Gesteland, R. F., *The RNA world: the nature of modern RNA suggests a prebiotic RNA world*. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1993; p xxiii, 630 p.
2. Joyce, G.; Orgel, L., Progress toward understanding the origins of the RNA world. *The RNA world* 2006, 23 - 56.
3. Bartel, D., Re-creating an RNA replicase. *The RNA world* 1998, 143 - 162.
4. Borowiec, J. A., 18 DNA Helicases. *Cold Spring Harbor Monograph Archive* 1996, *31*, 545-574.
5. Frankel, E., Dewey, D., Keating, C., Encapsulation of Organic Material in the Protocells. In *Astrobiology, An Evolutionary Approach*, Kolb, V., Ed. CRC Press: 2014.
6. Stage-Zimmermann, T. K.; Uhlenbeck, O. C., Hammerhead ribozyme kinetics. *RNA* 1998, *4* (8), 875-889.
7. Scott, W. G.; Finch, J. T.; Klug, A., The crystal structure of an aII-RNA hammerhead ribozyme: A proposed mechanism for RNA catalytic cleavage. *Cell* 1995, *81* (7), 991-1002.
8. Martick, M.; Scott, W. G., Tertiary contacts distant from the active site prime a ribozyme for catalysis. *Cell* 2006, *126* (2), 309-320.

P-27

HYPOXIA INHIBITS N^6 METHYLADENOSINE FORMATION IN mRNA

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Transcription and translation pathways have been well-studied, and their importance to gene expression is well understood. The importance of post-transcriptional regulation, however, is still not widely appreciated, nor well understood. One post-transcriptional pathway, mRNA degradation, has been a focus of our lab as mRNA stability can have dramatic effects on gene expression levels in response to a variety of conditions. Recently our lab has discovered that hypoxia, a condition known to play a role in tumor growth and angiogenesis, as well as heart disease and stroke, leads to an increase in the stability of a subset of ischemia related mRNAs. Identifying the underlying mechanisms behind mRNA stability has been difficult because many factors, including RNA binding proteins and miRNAs have been found to play a role in regulating mRNA stability. Recently, however, the mRNA modification N^6 methyladenosine has been shown target mRNAs for degradation. N^6 methyladenosine containing mRNA is bound by YTHD Family of RNA binding proteins which then transport the methylated mRNA to processing bodies where storage and degradation of mRNA occurs. Interestingly, our data suggests that N^6 methyladenosine mRNA levels are decreased under hypoxic conditions. We hypothesize that this decrease in mRNA methylation plays a role in increasing mRNA stability under hypoxic conditions, possibly through the increased expression of a known N^6 methyladenosine mRNA demethylase, ALKBH5. As such, ALKBH5 represents a potential therapeutic target for treatment of cancer as well as side effects from heart and muscle ischemia.

P-28

USING RECOMBINANT CAS9 RNPS TO ASSESS LOCUS MODIFICATION IN GENOME EDITING EXPERIMENTS

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Determining the extent to which specific loci are modified in genome editing experiments is important for characterizing tissues, mixed pools or isolated colonies of edited cells. This study compared *in vitro* digestion of PCR amplicons with recombinant Cas9 ribonucleoproteins (RNPs), to T7 Endonuclease I and other mismatch detection assays for determining the extent of locus modification in genome editing experiments.

We examined detection sensitivity, useful range of input, and activity on unpurified PCR products in targeting efficiency determination assays using model substrates that mimic insertion or deletion mutations. In addition, we compared these methods to high-throughput amplicon sequencing of libraries prepared from cell lines transfected with Cas9 and sgRNA targeting specific loci.

We find *in vitro* digestion with recombinant Cas9 RNPs to be a streamlined workflow for determining the extent of locus modification that enables the direct digestion and analysis of DNA without cleanup steps. *In vitro* digestion of PCR amplicons with Cas9 RNPs is as sensitive as mismatch detection assays for detecting indels. Unlike mismatch detection assays, Cas9 has the additional advantage of allowing for determination of targeting efficiencies above 50%. This is of value as targeting efficiency in genome editing experiments increases and for detection of biallelic editing in isolated cell colonies or tissues, and was previously only achievable using specialized PCR or amplicon sequencing approaches.

P-29

A RIBOSWITCH-CONTAINING sRNA CONTROLS GENE EXPRESSION BY SEQUESTRATION OF A RESPONSE REGULATOR

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Adenosyl cobalamine (AdoCbl) is required for the metabolism of ethanolamine (EA) in Enterococcus faecalis, both as an enzyme co-factor and for the induction of the EA utilization (eut) genes. It acts through an AdoCbl-binding riboswitch to induce the eut genes, but the mechanism of control is incompletely understood. Gene expression also requires EA, and this compound activates a two-component system composed of the sensor kinase, EutW, and its cognate response regulator, EutV, by phosphorylation. Active EutV is an antiterminator that binds nascent transcripts by recognizing a dual hairpin substrate and preventing terminator formation. In this study, we find that the AdoCbl-binding riboswitch is part of a small, trans-acting RNA, EutX, which additionally contains a dual hairpin substrate for EutV. In the absence of AdoCbl, EutX sequesters EutV in an inactive complex. When AdoCbl is present, its binding to the riboswitch prevents EutX/EutV complex formation, and EutV is free to induce gene expression by antitermination. These data therefore expand the known types of small RNA regulatory mechanisms to include riboswitch-mediated sequestration of RNA-binding regulatory proteins.

P-30

A UNIQUE HMG-BOX DOMAIN OF MOUSE MAELSTROM BINDS STRUCTURED RNA BUT NOT DOUBLE STRANDED DNA

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Piwi-interacting piRNAs are a major and essential class of small RNAs in the animal germ cells with a prominent role in transposon control. Efficient piRNA biogenesis and function require a cohort of proteins conserved throughout the animal kingdom. We studied Maelstrom (MAEL), which is essential for piRNA biogenesis and germ cell differentiation in flies and mice. MAEL contains a high mobility group (HMG)-box domain and a Maelstrom-specific domain with a presumptive RNase H-fold. We employed a combination of sequence analyses, structural and biochemical approaches to evaluate and compare nucleic acid binding of mouse MAEL HMG-box to that of canonical HMG-box domain proteins (SRY and HMGB1a). MAEL HMG-box failed to bind double-stranded (ds)DNA but bound to structured RNA. We also identified important roles of a novel cluster of arginine residues in MAEL HMG-box in these interactions. Cumulatively, our results suggest that the MAEL HMG-box domain may contribute to MAEL function in selective processing of retrotransposon RNA into piRNAs. In this regard, a cellular role of MAEL HMG-box domain is reminiscent of that of HMGB1 as a sentinel of immunogenic nucleic acids in the innate immune response.

P-31

microRNAs EXPRESSED BY ONCOGENIC *HERPESVIRUS SAIMIRI* TARGET HOST CELL-CYCLE AND IMMUNE REGULATORS

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Herpesvirus saimiri (HVS) is a T-lymphotropic g-herpesvirus that causes acute T-cell lymphomas and leukemias in New World primates. The most abundant transcripts in latently-infected marmoset T cells are seven non-coding U-rich RNAs, known as HSURs (*H. saimiri* U-rich RNAs). HVS also expresses six viral microRNAs (miRNAs), called hvs-miR-HSURs. Interestingly these HVS miRNAs are co-transcribed together with the HSURs as chimeric primary transcripts, and then further processed to give rise to mature forms.

Many viruses express miRNAs to regulate key gene expression programs in host cells, which benefit viral infection and life cycle. The functions of hvs-miR-HSURs are currently unknown. We hypothesized that the hvs-miR-HSURs might be a critical part of the viral gene expression program contributing to survival and propagation of the infected host cells during latency.

We used the Argonaute HITS-CLIP (High-Throughput Sequencing of RNA Isolated by Crosslinking Immunoprecipitation) method to identify potential host and viral mRNA targets of these hvs-miR-HSURs. The HITS-CLIP results revealed targets of hvs-miR-HSURs, including genes involved in the regulation of host cell cycle and anti-viral innate immune response. Gene Ontology (GO term) analyses of targets of hvs-miR-HSURs show enrichment of cell-cycle regulation, consistent with the T-cell oncogenic phenotype of HVS infection. Current efforts are underway to validate novel targets in infected T cells and to test their effects on cellular transformation and immune regulation.

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P-32

PROTEIN ARGININE METHYLTRANSFERASE 7 (PRMT7) PROMOTES BREAST CANCER CELL INVASION THROUGH THE INDUCTION OF MMP9 EXPRESSION

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Protein arginine methyltransferases (PRMTs) are a family of enzymes that modify proteins by methylating the guanidino nitrogen atoms of arginine residues. This methylation is important for the regulation of cellular processes such as chromatin remodelling, pre-mRNA splicing, and signal transduction. Many PRMTs are dysregulated in cancers and are capable of promoting tumourigenesis; thus, targeted therapies against these PRMTs could prove to be beneficial. Here, we focus specifically on PRMT7 whose mRNA expression has been shown previously in a genome-wide meta-analysis study to be correlated with breast cancer aggressiveness and metastasis. Our findings show that PRMT7 plays a role in breast cancer cell invasion by inducing the expression of matrix metalloproteinase 9 (MMP9). In invasive breast cancer tumours and cell lines, we noted a significant up-regulation of PRMT7 protein levels compared to normal breast tissues and breast epithelial cells. Interestingly, we observed two potential isoforms of PRMT7 which was never seen before in human cell lines. Surveys within *Ensembl* and NCBI databases predict these isoforms to be products of alternative splicing. Additionally, these isoforms seem to be differentially expressed in different breast cancer cell lines. Knockdown of both PRMT7 isoforms in highly invasive MDA-MB-231 and BT549 breast cancer cells resulted in morphological changes and decreased invasion through a Matrigel layer. Over-expression of PRMT7 in non-invasive MCF7 breast cancer cells promoted cell motility and invasion. We found that these effects were due to the positive regulation of MMP9. Currently, we are working on identifying the precise mechanism through which PRMT7 regulates MMP9 and determining the identity of the PRMT7 isoforms. The results of this study prove PRMT7 to be a promising therapeutic target in the treatment of invasive breast cancer.

P-33

SUPPRESSION OF PERVASIVE NON-CODING TRANSCRIPTION IN EMBRYONIC STEM CELLS BY esBAF

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Approximately 75% of the human genome is transcribed, the majority of which does not encode protein. However, most non-coding RNA (ncRNA) is rapidly degraded after transcription, and relatively few have established functions, questioning the significance of this observation. Here we show that esBAF, a SWI/SNF family nucleosome remodeling factor, suppresses transcription of ncRNAs from approximately 57,000 nucleosome-depleted regions (NDRs) throughout the genome of mouse embryonic stem cells (ESCs). We show that esBAF functions both to keep NDRs nucleosome-free and to promote elevated nucleosome occupancy adjacent to NDRs. Reduction of adjacent nucleosome occupancy upon esBAF depletion is strongly correlated with ncRNA expression, suggesting that flanking nucleosomes form a barrier to pervasive transcription. Upon forcing nucleosome occupancy near an NDR using a nucleosome-positioning sequence, we find that esBAF is no longer required to silence transcription. These data reveal a novel role for esBAF in suppressing pervasive transcription from open chromatin regions in ESCs.

P-34

INVESTIGATING THE ROLES OF ADHESION AND CYTOSKELETAL TENSION IN mRNA LOCALIZATION

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Cells create protrusions to aid in migration and guidance. These protrusions require specific proteins that might need to be synthesized locally. mRNAs play an important role in cellular migration and localize to protrusions during migration to aid in local protein synthesis. RNAs accumulate at sites of new integrin engagement, at lamellipodia, and at sites of early or persistent protrusion formation. In one pathway, the tumor suppressor protein, adenomatous polyposis coli (APC) targets RNAs to cell protrusions. APC associates with many RNAs in protrusive areas, including Ddr2, Rab13, and Pkp4. Most studies investigating mRNA localization have been performed on very rigid glass substrates. However, cells modify their spreading and migration behavior based on the extracellular matrix stiffness. Additionally, ECM stiffness varies throughout the body based on tissue type and location. Therefore, it is important to take into account the role of extracellular matrix stiffness and it's affect on mRNA localization.

The goal of this project is to determine how APC-associated mRNA localization changes on substrates of varying stiffnesss and dissect the mechanism of localization on the substrates. Using fluorescence in-situ hybridization (FISH) and polyacrylamide gels of varying stiffness, we were able to observe mRNA localization in fibroblasts. Using a method called the 'edge ratio' method, we were able to quantify mRNA localization. The edge ratio is a measure of the fraction of RNA in the periphery of the cell compared to the fraction of area in the cell periphery. This value is then compared to a control, or diffuse RNA. We observed that cells plated on stiff substrates (280kPa) had increased Ddr2 mRNA localization compared to soft (1kPa) substrates. Furthermore, we found that cells on constrained micropatterns had increased mRNA localization compared to freely spreading cells.

To dissect the mechanism of Dd2 localization, we hypothesized it was related to cytoskeletal tension and adhesion within the cell. Previous studies have shown that mechanical tension recruits mRNA to integrins. The identity or mechanism of this recruitment has not been studied. Immunostaining of actin, paxillin, tubulin, and de-tyrosinated tubulin was performed on the substrates of varying stiffness in addition to FISH. It was found that mRNAs were located at the tips of de-tyrosinated tubulin on stiffer substrates and expression of de-tyrosinated tubulin was reduced on soft substrates. In conclusion, this study investigates mRNA localization as a function of substrate stiffness. Cells alter their mRNA localization based on substrate stiffness, and this is related to cytoskeletal tension and adhesion.

P-35

ILLUMINATING CYCLIC DINUCLEOTIDE SIGNALING WITH RIBOSWITCH-BASED FLUORESCENT BIOSENSORS

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Most natural riboswitches sense primary metabolites and regulate genes in related biosynthetic pathways. Interesting exceptions to this rule are the different riboswitch classes that respond to the bacterial cyclic dinucleotide signaling molecules, cyclic di-GMP and cyclic di-AMP. Along with protein receptors, the riboswitches are responsible for transducing these cyclic dinucleotides, which are produced in response to primary or environmental cues, to regulate downstream cellular processes. For example, increases in cellular levels of cyclic di-GMP cause bacteria to transition from the motile to sessile, biofilm-forming stage.

Here I will present our strategy to adapt these riboswitches to generate selective, genetically-encodable fluorescent biosensors in order to elucidate novel stimuli and components of the signaling pathways. Using this approach, we have discovered a natural class of riboswitches that respond to the newfound cyclic dinucleotide, cyclic AMP-GMP (cAG, or 3', 3'-cGAMP). To our knowledge, these RNAs are the first specific receptors for this signaling molecule that have been identified. Progress towards understanding the structural basis for ligand specificity will be described, which is complementary to ongoing efforts to evolve a riboswitch to sense the mammalian cyclic dinucleotide, 2', 3'-cGAMP, that has an unusual 2'-5' linkage and is produced by the cytosolic DNA sensor cGAS. The discovery of a role for cyclic dinucleotides in innate immune signaling in mammals has implications for cancer immunotherapy, and so our development of fluorescent biosensors for these signaling molecules may be useful to researchers in this field.

P-36

A RpoS-REGULATED SMALL RNA THAT DAMPENS THE OSCILLATION OF GENES ENCODING ENZYMES FOR THE METABOLISM OF OXIDIZED NITROGEN COMPOUNDS

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Small regulatory RNAs (sRNAs) in bacteria play important roles in regulating metabolism in response to changing environmental conditions. The majority of these sRNAs act by base pairing with mRNA targets to affect their translation and/or stabilities. In *E. coli* and many other bacteria, base pairing requires the RNA chaperone Hfq. Here we describe a novel Hfq-binding sRNA that we have denoted RyeH. This sRNA is found in various strains of *E. coli* and *Shigella* and is encoded in the intergenic region between *sdiA*, encoding a transcriptional regulator of cell division and quorum-sensing, and *yecC*, encoding a hypothetical protein that may be a component of an ABC transporter.

Northern analysis showed that the *E. coli* RyeH RNA exists in two forms; short (~137 nt) and long (~178 nt). The two forms are transcribed from separate promoters but share the same Rho-independent terminator. The expression of *ryeH* is highest in late stationary phase. This induction is dependent on the stationary phase sigma factor, RpoS, since *ryeH* expression is greatly inhibited in the *rpoS* deletion strains. Our genetic screen from multicopy library identified that the overexpression of *rspA-ynfAB* represses RyeH. The functions of membrane protein YnfA and YnfB were unknown. However, RspA, a sugar dehydratase, was previously reported to prevent homoserine lactone-dependent synthesis of RpoS. In this study we found the overexpression of *rspA-ynfAB* inhibits RyeH in both RpoS-dependent and independent ways.

The observation that RyeH RNA co-immunoprecipitates with Hfq indicated that RyeH acts by basepairing with target mRNAs. Whole genome expression analysis revealed that *hmpA, narP* and *nfsA* mRNA levels are down regulated upon $RyeH_{short}$ overexpression. The *hmpA* gene encodes a nitric oxide dioxgenase and was found to oscillate in cells treated with nitric oxide-generating compounds. *nfsA* encodes a nitroreductase, which catalyzes the reduction of the nitrogroup (NO_2) in nitroaromatic compounds. *narP* encodes a nitrate/nitrite response regulator. Basepairing between $RyeH_{short}$ and the mRNAs was characterized by fusing wild type and mutant versions of the 5' UTR as well as first several codons of *hmpA, narP* and *nfsA* to chromosomal *lacZ* and monitoring the effect of wild type and mutant $RyeH_{short}$ overexpression. These assays confirmed that $RyeH_{short}$ base pairs with *hmpA, narP* and *nfsA* via two different sequences. Consistent with these observations, high levels of $RyeH_{short}$ conferred resistance to nitrofuran and azomycin, and the nitric oxide-induced *hmpA* oscillation is dampened by chromosomally-expressed RyeH. Based on these observations, we propose that RyeH contributes to the stationary phase adaptation to oxidized nitrogen compounds.

P-37

KSHV REGULATION OF THE microRNA BIOGENESIS PATHWAY

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Kaposi's sarcoma-associated herpesvirus (KSHV) is a γ-herpesvirus and the causative agent of Kaposi's sarcoma, the most common AIDS-associated malignancy in the US. We have identified MCP-1-induced protein-1 (MCPIP1), a critical regulator of immune homeostasis, as a highly down-regulated gene following *de novo* KSHV infection. MCPIP1 has recently been shown to suppress miRNA biosynthesis via cleavage of the terminal loops of precursor miRNAs. KSHV encodes for 12 pre-miRNAs that give rise to at least 18 mature viral miRNAs. We hypothesize that the host may benefit from MCPIP1 expression through MCPIP1-mediated repression of KSHV miRNA biogenesis of upon infection. Indeed, overexpression of MCPIP1 inhibits expression of KSHV miRNAs. However, KSHV works to circumvent the host immune reaction to expression of foreign miRNAs through the reduction of MCPIP1 levels, resulting in the expression of the KSHV viral miRNAs. Using KSHV miRNA mimics, we have identified KSHV miRNAs that target the 3'UTR of MCPIP1, resulting in decreased MCPIP1 expression. As opposed to MCPIP1, Dicer promotes miRNA biogenesis through the cleavage of precursor miRNAs and is involved in formation of the RNA-induced silencing complex (RISC). We have found increased expression of RISC components, Dicer and TRBP following *de novo* KSHV infection. Therefore, we see increased expression of a number of critical regulators of miRNA processing while expression of negative regulators of miRNA biogenesis is repressed. Our overall hypothesis is that the expression of KSHV miRNAs inhibit MCPIP1 and up-regulate Dicer, to evade host mechanisms of inhibiting expression of foreign miRNAs. KSHV-mediated alterations in global miRNA biogenesis represent a novel mechanism in which KSHV interacts with its host, and a novel mechanism for the regulation of miRNA expression.

P-38

THE TRANSCRIPTIONAL SILENCING PROTEIN SIR4 IS REQUIRED FOR Ku-MEDIATED TELOMERASE RECRUITMENT TO TELOMERES IN *SACCHAROMYCES CEREVISIAE*

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Telomeres are repetitive sequences at the end of eukaryotic linear chromosomes that buffer against shortening caused by the end-replication problem. To counteract this shortening, most organisms use the ribonucleoprotein enzyme telomerase to elongate telomeres. In *Saccharomyces cerevisiae* and humans, the RNA subunit of telomerase is bound by the Ku heterodimer, a complex best known for its role in DNA repair and also required for transcriptional silencing near telomeres in yeast. Ku binding to the yeast telomerase RNA, TLC1, promotes telomere lengthening and recruitment of telomerase to telomeres, but how Ku recruits telomerase to telomeres is not known. Ku has been reported to bind to the telomeric transcriptional silencing protein Sir4 *in vivo*, and we find that binding can be reproduced *in vitro*. We find that mutants lacking the *SIR4* gene, the Ku binding site in TLC1, or both all display very similar degrees of telomere shortening. These results could be explained if Ku recruits telomerase to telomeres by binding to telomere-associated Sir4. In further support of this hypothesis, we find that a TLC1 RNA containing three Ku-binding sites, TLC1(Ku)$_3$, causes telomere hyper-lengthening in wild type cells but not in *sir4*Δ cells. Chromatin immunoprecipitation experiments also show that deleting *SIR4* causes a decrease in telomerase recruitment to telomeres equivalent to that caused by disrupting Ku binding to TLC1. Furthermore, TLC1(Ku)$_3$ increases telomerase association with telomeres 10-fold in wild type cells, but this effect is abolished in *sir4*Δ cells. Lastly, to recapitulate recruitment of telomerase to telomeres via Sir4 *in vivo*, we tethered Sir4 directly to TLC1 RNA and found that tethering to a Ku-binding-defective TLC1 RNA restored telomeres to wild-type length. In summary, our results provide substantial evidence that Ku recruits telomerase to telomeres by binding Sir4, suggesting a previously unappreciated link between telomeric silent chromatin and telomerase regulation.

P-39

A FUNCTIONAL SCREEN IDENTIFIES microRNAs THAT INHIBIT DNA REPAIR AND SENSITIZE PROSTATE CANCER CELLS TO IONIZING RADIATION

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MicroRNAs have been implicated in DNA repair pathways through transcriptional responses to DNA damaging agents or through predicted microRNA regulation of DNA repair genes. We hypothesized that additional DNA damage regulating microRNAs could be identified by screening a library of 810 different human microRNA mimetics for induced changes in prostate cancer cellular sensitivity to ionizing radiation (IR). The results indicate that a large percentage of microRNAs increase sensitivity to IR, while a smaller percentage are IR protective. Two potent radiosensitizing microRNAs, miR-890 and miR-744-3p, significantly delayed radiation induced DNA damage repair and targeted multiple DNA repair genes including MAD2L2, WEE1 and RAD23B. Knock-down of miR-890 targets by siRNA was not sufficient to ablate miR-890 radiosensitization, signifying that miR-890 functions by regulating multiple DNA repair genes. Importantly, intratumoral delivery of miR-890, prior to IR therapy, significantly enhanced therapeutic effect. These results support miR-890 as a DNA repair modulating and radiosensitizing agent.

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P-40

THE USE OF A *DROSHA* NULL/CONDITIONAL-NULL MOUSE MODEL REVEALS RE-ANNOTATION OF LONG NON-CODING RNAs ACTING AS PRI-miRNA TRANSCRIPTS

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More than 45% of microRNAs (miRNAs) are derived from intergenic non-coding RNA transcripts and the characterization of their promoters is still considered as "elusive". The rapid cleavage of pri-miRNAs by Drosha hinders their identification with conventional techniques.

We present here an experimental, as well as a computational framework for high-throughput miRNA TSS identification. The former consists of a *Drosha null/conditional-null* (*Drosha*$^{LacZ/e4COIN}$) mouse model that has been generated using the novel conditional by inversion (COIN) methodology[1]. Whole transcriptome sequencing from mESCs derived from *Drosha*$^{LacZ/e4COIN}$ resulted to an extensive set of experimentally identified miRNA TSSs. This experimentally derived dataset was kept as an independent test set, and was utilized for the thorough evaluation of the computational method.

The later is an *in silico* approach that focuses on the identification of intergenic miRNA TSSs and relies on deeply sequenced RNA-Seq data. The algorithm called microTSS[2] integrates RNA-Seq data by creating "islands" of transcription (i.e. regions with increased RNA-Seq coverage) upstream of intergenic pre-miRNAs. The 5' end of each identified expressed region is treated as a putative TSS. This step is the backbone of the algorithm since it provides TSS candidates with single nucleotide resolution. A combination of three independent SVM models is subsequently utilized to score each candidate TSS and derive the final predictions. These SVM models have been trained on H3K4me3 and Pol II occupancy around protein coding TSSs, as well as on the existence of open chromatin domains, as identified by DNase-Seq.

microTSS was tested against TSSs identified in mouse using *Drosha null/conditional-null* mESCs, as well as TSSs detected using deeply sequenced global run-on sequencing (GRO-Seq) data in human IMR90 and ES cells. The described algorithm clearly outperforms other existing methodologies exhibiting more than 90% precision and sensitivity in all three investigated cell lines. The analysis of microTSS predictions in mES, hES and IMR90 cells showed that a significant number of pri-miRNAs overlap partially and in some cases completely with previously annotated lncRNAs. These findings suggest incomplete annotation of certain non-coding loci and/or multiple functionality. The analysis of GRO-Seq data unveiled a significant number of divergent pri-miRNAs upstream of protein coding gene promoters. The significantly smaller degree of conservation in these precursor sequences directly supports the proposed hypothesis[3] that divergent transcription is a model of new gene formation. MicroTSS is readily applicable to any cell line, tissue or organism and constitutes the missing part towards integrating the regulation of intergenic miRNA transcripts into the modeling of tissue specific regulatory networks.

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References:

1. Economides, A.N. et al. Conditionals by inversion provide a universal method for the generation of conditional alleles. *Proceedings of the National Academy of Sciences* Aug 20;110(34):E3179-88 (2013).
2. Georgakilas G, Vlachos IS, Paraskevopoulou MD, Yang P, Zhang Y, Economides AN, Hatzigeorgiou AG. microTSS: accurate microRNA transcription start site identification reveals a significant number of divergent pri-miRNAs. *Nat Commun*. 2014 Dec 10;5:5700.
3. Wu, X. & Sharp, P.A. Divergent transcription: a driving force for new gene origination? *Cell* 155, 990-996 (2013).

P-41

FIRST GENERATION OF NOVEL LaRNP INHIBITORS SENSITIZES HNSCC CELLS FOR CISPLATIN TREATMENT

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The human RNA-binding protein La is overexpressed in various types of tumor tissues, supports cell proliferation, motility and invasion of cancerous cells, increases tumor growth in mice and stimulates protein synthesis of target mRNAs encoding tumor-promoting factors such as cyclin D1, mdm2, and anti-apoptotic factor XIAP. Since siRNA-mediated depletion of La in cancerous cells significantly reduces both tumorigenesis and protein synthesis of tumor-promoting and anti-apoptotic factors, we hypothesized that hindering the binding of La to its target mRNAs might reduce expression of those factors and thereby represent a novel therapeutic approach.

In a first attempt toward this goal we aimed to develop a high-throughput fluorescence polarization assay allowing us to screen small compound libraries for molecules blocking the formation of ribonucleoprotein complexes (RNP) between the La protein and its target mRNAs, referred to as LaRNP inhibitors. After validation of primary hits in secondary assays, we tested those hits in cell-based assays. La stimulates the expression of the anti-apoptotic protein XIAP and our preliminary data show that La depletion by siRNA treatment or La inactivation by overexpression of a dominant-negative La mutant sensitizes cells for cisplatin treatment, suggesting that La stimulates an anti-apoptotic response. Hence, we asked whether inhibition of La by our first generation of LaRNP inhibitors increases cisplatin-induced cell death in head and neck squamous cell carcinoma (HNSCC) cells. Interestingly, treatment of HNSCC cells with a LaRNP inhibitor shifted the cisplatin IC_{50} toward lower cisplatin concentrations. These initial data are strongly supporting the aim of our NCI-funded project to screen for LaRNP inhibitors and to further develop those molecules into potent inhibitors applicable in novel anti-cancer combination treatment strategies.

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BUILDING AN INTERACTION NETWORK OF NUCLEAR ARGONAUTE 2

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RNA interference (RNAi) is an endogenous mechanism for regulating gene expression that can be manipulated for experimental or therapeutic purposes to knockdown protein expression. In the mammalian cell cytoplasm, small RNAs direct protein machinery to cleave mRNA targets. Argonaute 2 (Ago2) is the core catalytic protein of RNAi that possesses target cleavage function. Recently, Ago2 and the RNAi machinery were shown to function in the nucleus of human cells (Gagnon et al, 2014). Ago2 uses promoter-targeted duplex RNAs to induce transcriptional silencing and activation through the RNAi pathway (Chu et al. 2010, Matsui et al. 2013). Additionally, Ago2 was also shown to direct duplex RNAs to redirect alternative splicing (Liu et al. 2012). Although cytoplasmic RNAi processes have been extensively studied, the mechanisms of RNAi and roles for Ago2 within the nucleus are not well understood.

To better understand nuclear gene regulation via RNAi, our goal was to build a protein interaction network of nuclear Ago2 by utilizing semi-quantitative (SINQ) mass spectrometry analysis. To do this, we used three different levels of analysis: 1) FLAG-Ago2 vs. untagged Ago2. 2) Cytoplasmic vs. nuclear Ago2 3) RNase vs. Untreated Ago2. To carry out these analyses, we prepared cytoplasmic and nuclear cell extracts and performed large-scale Ago2 immunoprecipitations for mass spectrometry analysis of protein complexes. From this protocol, we compiled a list of proteins that were consistent and significant across triplicate samples in all levels of analysis.

The RNase treatment weakened detection of TRBP in the cytoplasm, supporting the fact that it interacts with Ago2 in an RNA-dependent matter. The overlap between the FLAG-Ago2 and untagged Ago2 interactions was primarily RNAi factors. The overlap between the cytoplasmic and nuclear Ago2 interactions was exclusively RNAi factors, supporting the role of RNAi in the nucleus. The protein interactions unique to nuclear Ago2 included factors involved in nuclear import, splicing, and histone methylation, suggesting new roles for Ago2 regulation and function in the nucleus.

We will perform co-immunoprecipitations to verify the novel interacting factors. The future directions for this project are to study interactions more in-depth to provide a clear understanding of the mechanisms by which Ago2 functions in nuclear gene regulation.

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MODULATION OF T BOX RIBOSWITCH EFFICACY BY SMALL MOLECULES

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Riboswitches are intriguing potential targets for the development of novel antibacterial agents. The T box riboswitch, found primarily in Gram-positive bacteria, regulates expression of many essential genes by selectively responding to cognate, uncharged tRNA to control transcription readthrough or translation initiation. The effect of molecular crowding on the *Bacillus subtilis glyQS* T box riboswitch and molecular recognition by small molecules was investigated using a multi-round fluorescence monitored *in vitro* transcription assay. The results indicate that small molecules and co-factor/co-solvent additives modulate the T box riboswitch tRNA affinity and transcription readthrough efficacy and have significant implications for riboswitch-targeted drug discovery and structure-function studies.

P-44

A LONG NON-CODING RNA INDUCED BY PARTIAL HEPATECTOMY NEGATIVELY MODULATES HEPATOCYTE PROLIFERATION DURING LIVER REGENERATION

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To characterize the roles of long non-coding RNAs (lncRNAs) in cell proliferation, we analysed lncRNA expression in a mouse partial hepatectomy (PHx) model, in which synchronized cell proliferation is precisely regulated in response to the loss of liver mass. Expression of more than 400 lncRNAs was changed during this process. Of these, long non-coding RNA induced by partial hepatectomy 2 (LncPHx2), was highly upregulated during liver regeneration. Depletion of LncPHx2 from regenerating liver by antisense oligonucleotide led to faster liver regeneration. Gene expression analysis showed that LncPHx2 depletion resulted in upregulation of mRNAs encoding proteins known to promote cell proliferation, including MCM components, DNA polymerases, histone proteins, and transcription factors. LncPHx2 interacts with the mRNAs of MCM components in cultured cells, indicating it might regulate the expression of MCMs and other genes post-transcriptionally. Collectively, we demonstrate a novel negative feedback regulation involving a lncRNA in regulating hepatocyte proliferation during liver regeneration.

P-45

SELENIUM ATOM-SPECIFIC FUNCTIONALIZATION OF RNA FOR STRUCTURE AND FUNCTION STUDIES

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There are total five essential elements (H, C, N, O and P) in nucleic acids. Atom-specifically functionalized RNAs by introducing the sixth element can offer nucleic acids many unique and novel properties without significant perturbation of three-dimensional structures and structural features of nucleic acids and their protein complexes. Single atom replacement (or atom-specific mutagenesis) of nucleic acids means the substitution of an atom (such as O) with another atom from the same elemental family (such as S, Se and Te). Since RNAs can involve in complex biological processes as regulators, RNA diversities in both function and structure have been greatly appreciated. RNA possesses not only the ability to store genetic information and participate in transcription and translation, but also the capacity to adopt well-defined three-dimensional structures, which can be readily adjusted to meet various functional needs (such as catalysis). Although the importance of numerous RNAs in catalysis, gene expression, protein binding and therapeutics has been acknowledged by the entire scientific society, current understanding of RNA function and structure is still limited. Thus, atom-specific mutagenesis of RNA provides useful tools to investigate nucleic acid folding and catalysis, study nucleic acids and their protein interactions, improve biochemical and biophysical properties of nucleic acids, facilitate gene silencing and delivery in drug discovery and RNA and DNA nanotechnology, and explore potential nucleic acid therapeutics. Our presentation will focus on the most recent selenium-atom functionalization of RNA and their potential applications in 3D structure-and-function studies and as anticancer therapeutics in molecular medicine.

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Selected Publications:

1. Rob Abdur, Oksana O. Gerlits, Jianhua Gan, Jiansheng Jiang, Jozef Salon, Andrey Y. Kovalevsky, Alexander Chumanevich, Irene T. Weber, Zhen Huang*, “Novel Complex MAD Phasing and RNase H Structural Insights by Selenium Oligonucleotides”, 2014, *Acta Crystallographica Section D*, 2014, *D70*, 354-361.
2. Jia Sheng, Jianhua Gan, Alexie Soars, Jozef Salon and Zhen Huang*, “Structural Insights of Non-canonical U•U Pair and Hoogsteen Interaction Probed with Se Atom”, 2013, *Nucleic Acids Research*, *41*, 10476-10487.
3. Huiyan Sun, Sibo Jiang, Julianne Caton-Williams, Hehua Liu, and Zhen Huang*, “2-Selenouridine Triphosphate Synthesis and Se-RNA Transcription”, *RNA*, 2013, *19*, 1309-1314.
4. Jozef Salon, Jianhua Gan, Rob Abdur, Hehua Liu and Zhen Huang*, “Synthesis of 6-Se-Guanosine RNAs for Structural Study”, *Organic Letter*, 2013, *15*, 3934-3937.
5. Wen Zhang, Abdalla E. Hassan, and Zhen Huang*, “Synthesis of Novel Di-Se-containing Thymidine and Se-DNAs for Structure and Function Studies”, *Science China: Chemistry*, 2013, *56*, 273-278.
6. 2. Huiyan Sun, Jia Sheng, Abdalla E. A. Hassan, Sibo Jiang, Jianhua Gan and Zhen Huang*, “Novel RNA Base Pair with Higher Specificity using Single Selenium Atom”, *Nucleic Acids Res.*, 2012, *40*, 5171-5179.
7. Jia Sheng, Wen Zhang, Abdalla E. A. Hassan, Jianhua Gan, Alexei Soares, Song Geng, Yi Ren, Zhen Huang*, "Hydrogen Bond Formation between the Naturally Modified Nucleobase and Phosphate Backbone", Nucleic Acids Research, 2012, *40*, 8111-8118.
8. Wen Zhang, Jia Sheng, Abdalla E. Hassan, and Zhen Huang*, "Synthesis of Novel 2’-Deoxy-5-(Methylselenyl)Cytidine and Se-DNAs for Structure and Function Studies", *Chemistry-An Asian Journal*, 2012, *7*, 476-479.
9. Lin, L.; Sheng, J.; Huang, Z. *Chemical Society Reviews*, 2011, 40, 4591.
10. Sheng, J.; Hassan, A. ; Zhang, W.; Zhou, J.; Xu, B.; Soares, A. S.; Huang, Z. *Nucleic Acids Res.*, 2011, 39, 3962.
11. Caton-Williams, J.; Lin, L.; Smith, M.; Huang, Z. *Chemical Communications*, 2011, 47, 8142.
12. Abdalla E. A. Hassan, Jia Sheng, Wen Zhang, and Zhen Huang*, "High Fidelity of Base Paring by 2-Selenothymidine in DNA", *Journal of American Chemical Society*, 2010, *132*, 2120-2121.

P-46

USING HYDROXYL RADICAL FOOTPRINTING TO OBSERVE RIBOSOME ASSEMBLY INTERMEDIATES *IN VIVO*

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The assembly of the *E. coli* ribosome small subunit has been widely studied and characterized *in vitro*. Despite this, ribosome biogenesis in living cells remains poorly understood. This is a very complex process in which an rRNA is transcribed, folded, cleaved, and modified, while also binding with 20 different proteins. Very little is known about how the tertiary structure of the ribosomal RNA changes during assembly. There are a number of structure-probing methods that can be used to study rRNA *in vivo*, but virtually all of them lack the time resolution necessary to study a process like ribosome synthesis, which is completed within a few minutes.

Hydroxyl radical footprinting can be used to probe *in vivo* rRNA structure. The hydroxyl radicals which probe the rRNA can be produced in milliseconds using synchrotron X-rays. With this technique it is possible to examine ribosome assembly with meaningful time resolution. The hydroxyl radicals cleave the RNA backbone in solvent accessible regions. In this way, the cleavage pattern can be used to gain information on regions of flexibility and rigidity within an RNA.

Once the RNA in a cell has been irradiated, it is necessary to isolate the modified RNA. For the purpose of examining ribosome assembly, it is nascent ribosomes that are of interest, not pre-existing ribosomes that are already assembled. Therefore, the nascent ribosomes must be isolated from the background of pre-existing ribosomes. It has been shown that cells can take up labeled nucleosides that have been added to their growth media and incorporate them into nascent RNA transcripts. These can then be isolated using affinity methods. Once the nascent, assembling rRNA has been isolated, it can be analyzed by primer extension. The reverse transcriptase terminates at the cleavage sites. The cDNA fragments can then be analyzed by slab gel, capillary electrophoresis, or high-throughput sequencing methods.

P-47

TRANSCRIPTIONAL PAUSES AND ERRORS FREQUENTLY OCCUR AT CpG SEQUENCES IN *E. COLI*

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It has been proposed that transcription errors destabilize genomes by inducing pausing of RNA polymerase. Genome-wide sequencing of the nascent-transcripts when combined with RNase footprinting reveals that transcriptional pauses and errors primarily occur at CpG sequences in *E. coli*.

P-48

AMPHIPHATIC DNA-BASED TAPS (dTtaPS) FOR THE DELIVERY OF UNCHARGED NUCLEIC ACIDS (PNA AND/OR PMO)

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An innovative approach to the delivery of uncharged peptide nucleic acids (PNA) or phosphorodiamidate morpholino (PMO) oligomers into mammalian cells is proposed and consists of extending the sequence of these oligomers with a short (6-mer) PNA-polyA or PMO-polyA tail. Recognition of the polyA-tailed PNA or PMO oligomers by an 8-mer amphipathic trans-acting polythymidylic thiophosphate triester element (dTtaPS) results in efficient internalization of these oligomers into various cell lines. The functionality of the internalized oligomers is demonstrated through alternate splicing of the pre-mRNA luciferase gene in HeLa pLuc 705 cells. Amphipathic phosphorothioate DNA elements represent a novel class of cellular transporters for efficient delivery of uncharged nucleic acid sequences in live mammalian cells.

P-49

MESENCHYMAL STEM CELLS ENGINEERING WITH mRNA-ITGA4 AS CLINICALLY APPLICABLE mRNA-BASED TOOL FOR INTRA-ARTERIAL BRAIN TARGETING

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There is growing interest in the use of mesenchymal stem cells (MSCs) in the rapidly expanding field of regenerative medicine. MSCs are characterized by a wealth of trophic factors and immunomodulatory properties which were shown to have a therapeutic potential for brain diseases. However, the delivery methods to the brain still need to be optimized. Intra-arterial route has been found clinically very attractive, but it requires that MSCs needs to be equipped with mechanisms allowing for diapedesis from the vessel lumen to the brain parenchyma. We have already shown in a model stem cell line that a mechanism borrowed from the leukocytes such as integrin expression may be effective. However, there is enormous resistance of primary MSCs to the traditional, plasmid-based methods of transfection. Thus, we investigated mRNA as a potential vehicle for induction of expression of integrin genes.

Initially, we evaluated mRNA-based protein synthesis using commercial mRNA-GFP (Stemgent). We observed robust and intensive GFP signal, which lasted at least one week thus we found that mRNA-based technology is potentially useful for gene expression induction. For integrin expression the cDNA of ITGA4 gene was cloned into pSP72 vector under T7 promoter (P2191-Promega) and utilized as a template for mRNA production *in vitro*. T7 mMessage mMachine Kit (AM1344,-Ambion) with poly(A) tailing kit (AM1350-Ambion) and mMessage mMachine ® T7 Ultra Kit (AM1345-Ambion) including ARCA cap analogue were used. For mRNA stabilization, SSB protein (S3917-Sigma) was employed. Human MSCs (PT2501-Lonza) and HEK293 control cells were transfected with mRNA-ITGA4 by Lipofectamine ® 2000 (Invitrogen). RT-PCRs were carried out to assess transfection efficacy. Immunocytochemistry assays were performed to confirm the exogenous ITGA4 protein synthesis.

An effective *in vitro* production of mRNA-ITGA4 with posterior efficient cellular delivery were achieved with T7 mMessage mMachine Kit, but ITGA4 protein was absent in both MSCs and HEK293 cells. SSB protein stabilization effect on mRNA-ITGA4 resulted in an effective ITGA4 protein synthesis in HEK293 cells, whereas the ITGA4 protein in MSCs were undetectable. The ITGA4 protein synthesis was achieved in MSCs after transfection with mRNA-ITGA4 containing anti-reverse-cap-analogue (ARCA). The ITGA4 protein presence in transfected MSCs was transient and changed its location from inner cellular structures toward membranes where up to 24h lasted. The additional experiments have shown that delivered mRNA-ITGA4 was present inside the transfected cells much longer (at least one week), but its translation was absent.

In this study, we have shown that mRNA-based expression of integrin gene is feasible and the use of ARCA to produce exogenous mRNA appear to increase translation efficiency. While being short-term (24 h) it should be sufficient for brain targeting after intra-arterial delivery. The shorter expression window of integrin gene in comparison to GFP was not explained yet, but we speculate that integrins which are native human cell proteins may be subjected to internal down regulation such as by microRNA, while GFP being not native human protein may be able to escape internal regulatory mechanisms. Our study shows that not transfection, but translation is a major obstacle in pursuing mRNA-based protein expression in MSCs.

P-50

EVIDENCE FOR CO-TRANSCRIPTIONAL PACKAGING OF GENOMIC RNA BY THE RETROVIRAL GAG PROTEIN

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Retroviruses serve as a model system for studying nuclear export and subcellular trafficking of unspliced viral RNA, which they use as both a template for protein synthesis (mRNA) and as their viral genome (gRNA). Retroviruses depend upon both species of unspliced viral RNAs for replication and must overcome cellular roadblocks that prevent the export of unspliced RNAs from the nucleus. Complex retroviruses, such as HIV, encode accessory proteins (Rev) that facilitate the nuclear export of unspliced viral RNA. Simple retroviruses, including the avian retrovirus Rous sarcoma virus (RSV), do not encode Rev-like proteins, so the mechanism of unspliced viral RNA export is still under study.

To assemble new virus particles in an infected cells, the retroviral structural polyprotein Gag specifically selects unspliced gRNA for encapsidation into new virus particles through a high-affinity interaction with the psi packaging sequence in the 5' untranslated region. Previously, it was thought that Gag functioned solely in the cytoplasm to facilitate virus assembly. However, we discovered that RSV Gag transiently traffics through the nucleus using Crm1 as a nuclear export cofactor. Although there are many potential roles for Gag in the nucleus, we have generated genetic and biochemical evidence that RSV Gag may select its gRNA in the nucleus for subsequent encapsidation into virus particles.

To examine this possibility more directly, we created an infectious RSV provirus that contains 24 copies of RNA stem loops from the MS2 bacteriophage (RSV-24x). When coexpressed with the MS2 coat protein fused to YFP, RSV-24x RNA appears as foci in the nucleus, cytoplasm, and along the plasma membrane, the site of virus particle release. To visualize the Gag protein simultaneously, we expressed WT Gag-GFP in *trans*, and using confocal live cell time-lapse microscopy, we observed WT Gag co-localized with viral RNA in distinct foci in the nucleus. Because the amount of WT Gag in the nucleus is very low, we used a mutant of Gag deficient in nuclear export (Gag.L219A) that accumulates in the nucleus and localizes to distinct subnuclear foci. When coexpressed with Gag.L219A-GFP, RSV-24x RNA colocalizes with the Gag mutant in nuclear foci at a high frequency. A reduced level of Gag:RNA colocalization was observed when the psi packaging sequence was deleted, suggesting specificity of the interaction. Because the formation of Gag foci was dependent on ongoing RNA transcription, and Gag.L219A associates at a low level with newly synthesized cellular RNA, we hypothesize that Gag may bind RNA co-transcriptionally. Together, these data suggest that RSV Gag may traffic to sites of active transcription to sample nascent RNAs using a "scan and lock" mechanism in search of the high affinity psi packaging sequence.

To determine whether the Gag proteins of other retroviruses traffic to sites of viral RNA synthesis, we created a stable cell line that contains a dox-inducible, noninfectious HIV genome that produces Gag-GFP. We utilized fluorescence *in situ* hybridization to specifically label unspliced HIV RNA. Using confocal microscopy, we observed foci of Gag within the nucleus, where a subpopulation was colocalized with the unspliced viral RNA at sites of transcription. Treatment of cells with transcription inhibitors diminished the number of Gag and viral RNA nuclear foci. In addition, when cells were treated with the Crm1 inhibitor leptomycin B, the number of nuclear Gag foci was greatly reduced, suggesting that HIV Gag may traffic to sites of viral RNA synthesis in a Crm1-dependent manner. Together, these data suggest that nuclear trafficking of retroviral Gag proteins and their association with unspliced viral RNA at sites of transcription may be a conserved mechanism for selection of gRNA as a way to bind the unspliced viral RNA before it becomes spliced or exported into the cytoplasm as an mRNA.

P-51

REGULATION OF AXONAL TRAFFICKING OF NUCLEAR-ENCODED MITOCHONDRIAL COXIV mRNA

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It is now well-established that a distinct subset of nuclear-encoded mitochondrial mRNAs such as Cytochrome C oxidase IV (COXIV), are selectively transported and translated in the distal structural/functional domains of neurons (i.e. axons and presynaptic terminals). Local translation of the COXIV mRNA, which encodes a key subunit of oxidative phosphorylation complex IV, plays an important role in axonal energy metabolism, function and growth. Disruption of local COXIV expression leads to compromised mitochondrial membrane potential, decreased ATP levels and generation of reactive oxygen species (ROS) in the axon. However, relatively little is known about the mechanisms involved in regulating the axonal trafficking of these nuclear-encoded mitochondrial transcripts.

In previous studies, we identified a putative 38-nucleotide stem-loop structure (zipcode) in the 3'-untranslated region of the COXIV transcript that was necessary and sufficient for the axonal localization of COXIV mRNA in superior cervical ganglion (SCG) neurons. To identify proteins involved in the axonal transport of the COXIV transcript, we used this 38-nucleotide COXIV RNA zip-code as bait for RNA-protein binding studies. Gel-shift assays of the biotinylated COXIV-zipcode incubated with retinoic-acid differentiated SHSY5Y cell lysates showed that the zipcode binds endogenous proteins and forms nucleoprotein complexes. Mass spectrometric analysis of proteins isolated by affinity purification using biotinlylated COXIV zipcode oligomer incubated with SHSY5Y cell-lysates lead to the identification of a number of RNA-binding and mitochondria-associated proteins such as fused in sarcoma/translated in liposarcoma (FUS/TLS) and Parkinson disease protein 7 (PARK7/DJ-1) respectively. Validation using western blotting analyses confirmed the presence of the candidate proteins in the COXIV-zipcode affinity purified complexes from SHSY5Y lysates. In addition, using immunohistochemical and western blotting analyses, we also established the presence of these candidate COXIV-zipcode binding proteins in SCG neurons. Future experiments are aimed at confirming the interaction of these candidate binding proteins with the endogenous COXIV mRNA in SCG neurons and assessing the *in vivo* significance of the candidate proteins in axonal transport of COXIV mRNA.

P-52

COMPARISON OF CRYSTAL STRUCTURES AND *IN VITRO* STUDIES GIVE AN INSIGHT OF THE EVOLUTION OF PROTEINACIOUS RNase Ps IN *ARABIDOPSIS THALIANA*

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Protein-only RNase Ps (PRORPs) represent a new class of enzymes that cleave the 5' end of premature tRNAs in Eukaryotes. *Arabidopsis thaliana* PRORP1 and PRORP2 enzymes are both nuclear encoded but have different cellular localization; PRORP2 localizes to the nucleus, PRORP1 to the chloroplast and the mitochondria. Here we were able to capture a snapshot of the early diversification of these *A. thaliana* PRORPs by comparing *in vitro* functional data and the crystal structures of PRORP1 and PRORP2. We obtained a 3.3 Å resolution crystal structure of PRORP2 and compared it to the previously published structure of PRORP1. Both of the proteins have an overall V-shape with similar structural features; however PRORP2 adopts a more open conformation than PRORP1. *In vitro* properties of PRORP1 and PRORP2 also show high similarity. Mg2+ dependent activities under single turnover conditions are comparable (k_{obs} = 0.2-1 min-1) for nuclear and organellar substrates, although PRORP2 processes mitochondrial substrates slightly slower than nuclear ones. Mutational analysis in the metallonuclease domain of PRORP2 reinforces the importance of conserved aspartate and histidine residues involved in catalysis. In summary, the overall similarities but minor differences of PRORP1 and PRORP2 suggest that these enzymes are in the early steps of specialization after localization into different cellular compartments.

P-53

A RIBOSOMAL FRAMESHIFTING STRUCTURE IN THE CCR5 mRNA LEADS TO miRNA-STIMULATED NONSENSE-MEDIATED mRNA DECAY

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RNA structure-based signals are used by viruses to affect programmed ribosomal frameshifting (PRF) yielding extended fusion proteins in a fraction of translation processes. Computationally identified -1 PRF signals in cellular mRNAs have been predicted to lead to premature termination of translation in the vast majority of cases (in excess of 99% of all the cases). We found that a -1 PRF signal predicted in the mRNA of the human chemokine receptor type 5 (CCR5) is a pseudoknotted structure that is responsible for redirecting translation toward a premature termination codon (PTC), ultimately destabilizing the mRNA via the nonsense mediated mRNA decay pathway (NMD) and possibly another decay pathway. CCR5 is also a co-receptor used by HIV-1 to enter its target CD4+ T-cells. We built a 3D model of the -1 PRF structure and validated its stability in molecular dynamics simulations (MD). The structure is a two-stem pseudoknot, with the larger of the two stem domains consisting of multiple half-turn helical segments, separated by asymmetric single strands that enable bending of the larger stem region and ultimately bridging of the two stems. MD predicted this larger region and the 3' end of the structure to be very stable, consistent with the experimental SHAPE data and the idea that it may form triple base interactions with miR-1224. Such interactions can increase the stability of the whole -1 PRF structure, explaining the experimentally observed increase in the fraction of paused ribosomes. Another miRNA, miR-141, is also predicted to interact with the same region. Experiments have also demonstrated that several other cytokine receptor mRNA -1 PRF signals are controlled via miRNAs. Altogether these results indicate a novel mechanism of cellular gene expression regulation via a -1 PRF structural signal, modulated further by the sequence-specific miRNA interactions.

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P-54

CD47 MODULATE microRNA ON EXOSOMES IN CELL SPECIFIC MANNER

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Exosomes (EVs) have been capturing great attention due to their role in cell-cell communication at cellular levels by transferring functional mRNA, miRNA, non-coding RNA through specific heterogeneous nuclear ribonucleoproteins (HNRNPs) to target recipient cells. Recently, we have discovered that the CD47 receptor of Thrombospondin 1 plays an important role in Jurkat T cell and endothelial communications by altering the global gene expression of recipient HUVEC cells as well as angiogenic VEGFR2 pathways in CD47 dependent manner. We further performed Gene enrichment analysis of HUVECs treated with T cell derived EVS which showed enrichment of T cell signaling. To further elucidate this mechanism, we performed global genomic cellular mRNA expression profile of WT parental Jurkat and Jinb8 T cells and their EVs mRNAs and found that 590 and 178 transcripts were differential expressed between Jurkat and JINB8 respectively. Although, these EVs had very little mRNA and majority of the mRNA was non-coding RNAs which are consistent with literature that EVs are enriched with miRNA and non-coding RNA. To confirm this, we performed genomic miRNA microarray and found 257 microRNAs differentially expressed between Jurkat and Jinb8 T cells. These results were further confirmed using CD47-siRNA in Jurkat cells and found 95 miRNA are CD47 dependent. However, miRNA profile from EVs derived from WT and Jinb8 T cells were different from their cellular parental cell line miroRNAs which is also consistent with Mittelbrunn et al (Nat Commun. 2011; 2:282). Using MEME and JASPAR Bioinformatics and computational analysis of miRNA predicted that Jinb8 EVs microRNA are enriched for 3 motifs (AGAAAA, GGGG and GGGAAGG) and Jurkat EVs microRNAs showed an enrichment for a single motif (AGGCAG) that may be involved in miRNA sequence recognition or regulation. To investigate CD47 dependent differential miRNAs sorting into exosomes through binding to specific microRNA motifs, we performed proteomic analysis and found differential expression of HNRNPD and HNRNPAB between EVs derived from WT and Jinb8. We currently are validating our miRNA and proteomics results using real time PCR, flow cytometry and Western blot analysis. This suggest that the presence of CD47 at the surface of T cells alters miRNA expression and may have role in miRNA sorting on T cell derived EVs through specific protein and RNA motifs.

P-55

ROLE OF THE INSULIN 5'UTR BINDING FACTORS IN INSULIN GENE REGULATION

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Insulin regulates the glucose homeostasis in mammals and its biosynthesis is regulated by glucose. In the initial phase of glucose stimulation, insulin biosynthesis is regulated mainly at the translation level. 5'UTR of the insulin mRNA plays important role in insulin translation regulation where some of the trans-acting factors from β-Cells of the pancreas bind to it and up-regulate the insulin translation. Previously we have identified Protein Disulfide Isomerase (PDI), Poly A binding protein (PABP) and pancreatic amylase as a one of the insulin 5'UTR-binding trans-acting proteins by biotin RNA pull down assay followed by mass spectrometry. We have shown that upon glucose stimulation, PDI (Protein disulfide isomerase) gets phosphorylated and the activated PDI reduce the disulfide bonds of the PABP and forms a complex with insulin mRNA (5'UTR) to increase insulin biosynthesis. Phosphatase treatment of the extract decreases the binding activity suggesting that phosphorylation of PDI is required for its binding to insulin mRNA. We believe that upon glucose stimulation specific kinase gets activated and phosphorylate PDI which leads to increase in insulin biosynthesis.

Pancreatic amylase is the other protein which we have identified in the insulin 5'UTR-binding complex and the Insulin 5'UTR binding activity of amylase was confirmed by gel shift assay, Immunodepletion assay and yeast 3 hybrid assay. Bacterially over-expressed and affinity purified recombinant amylase is also specifically binding with insulin 5'UTR. Although pancreatic amylase is not expressing in β-cells of the islets in normal conditions but the high level of Th1 cytokines (TNF-α and IFN-γ) induces the amylase expression in β-cells which eventually reduce the insulin production. There are reports which is showing that patients with chronic or acute pancreatitis disease having high level of Th1 cytokines in pancreatic β-cell. So we hypothesize that this leads to amylase induction followed by loss of insulin production and eventually to apoptosis and diabetes in these patient.

P-56

EXAMINATION OF TARGET TRANSCRIPTS OF THE CIRCADIAN DEADENYLASE *NOCTURNIN* BY POLY(A)DENYLOME ANALYSIS

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Poly(A) tails are modifications of mRNAs that are important for the control of mRNA stability and translation initiation, and therefore enzymes that regulate the poly(A) tail length, such as polyadenylases and deadenylases, can significantly impact the ultimate protein profiles in the cell. Since each deadenylase differs in its tissue distribution and cellular localization, it has been hypothesized that they may target specific transcripts, although this has not been clearly demonstrated. Among eleven putative deadenylases in mammals, *Nocturnin* (*Noc*) is unique, as its expression is robustly rhythmic, peaking during the night. Loss of *Noc* in mice (*Noc* KO) results in resistance to diet-induced obesity but the identity of the target mRNAs that cause this phenotype are not known. In order to identify NOC target transcripts, we utilized our recently developed technique, Poly(A)denylome analysis, to globally identify mRNAs that have altered poly(A) tail lengths in *Noc* KO mouse livers as compared to WT. We identified 213 transcripts that have extended poly(A) tails in *Noc* KO tissue, making them strong candidates for NOC target mRNAs. However, examination of the properties of these transcripts revealed that the length of the poly(A) tails did not correlate with changes in mRNA abundance, and these putative targets were generally not rhythmic at the steady-state level. We also identified transcripts whose mRNA expression levels were significantly changed in the *Noc* KO livers but this group has only modest overlap with those that have altered poly(A) tail lengths, suggesting that these are indirectly affected by loss of NOC. However, gene ontology and network analyses show that both of these groups are significantly enriched in mRNAs with roles in ribosome function and translation and in mitochondrial oxidative phosphorylation. These results indicate a novel and complex role for NOC in regulating these critical biological pathways.

P-57

RIBOSOMES SLIDE ON LYSINE-ENCODING HOMOPOLYMERIC A STRETCHES

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Protein output from synonymous codons is thought to be equivalent if appropriate tRNAs are sufficiently abundant. Here we show that mRNAs encoding iterated lysine codons, AAA or AAG, differentially impact protein synthesis: insertion of iterated AAA codons into an ORF diminishes protein expression more than insertion of synonymous AAG codons. Kinetic studies in E. coli reveal that differential protein production results from initial pausing on consecutive AAA-lysines followed by ribosome sliding on homopolymeric A sequence. Translation in a cell free-expression system demonstrates that diminished output from AAA-codon-containing reporters results from premature translation termination on out of frame stop codons following ribosome sliding. In eukaryotes, these premature termination events target the mRNAs for nonsense-mediated decay. The finding that ribosomes slide on homopolymeric A sequences explains bioinformatic analyses indicating that consecutive AAA codons are underrepresented in gene-coding sequences. Ribosome 'sliding' represents an unexpected type of ribosome movement possible during translation.

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FUNCTIONAL SCREENING OF AR SIGNALING AXIS IDENTIFIES NOVEL AR-REGULATING microRNA

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Background: The androgen-receptor (AR) is a steroid receptor and transcription factor that is active and expressed in most all stages of prostate cancer (PCa) progression, including in castration resistance (CRPC). The AR is the primary therapeutic target for metastatic PCa through androgen deprivation or androgen blockade therapies; however, resistance is readily acquired through aberrant androgen production, AR gene mutation, AR gene amplification, or alternate AR mRNA splicing. In light of this, the AR signaling pathway remains to be a major point of focus in PCa biology and PCa therapeutics. To further characterize the regulatory mechanisms of AR expression and activity we sought to identify microRNAs (miRNAs) that influence the AR signaling axis. miRNAs are a potent class of non-coding RNAs which regulate gene expression through sequence-specific binding to coding gene mRNAs. There are limitations to the bioinformatic prediction of miRNA targets; therefore, it remains challenging to forecast miRNAs which regulate a complex pathway, such as AR signaling. To circumvent this we applied a series of high-throughput functional screens, using a miRNA mimetic library, to identify miRNAs that regulate the AR and AR signaling in PCa.

Methods: More than 800 human miRNA mimetics were co-transfected into LNCaP cells with an AR transcriptional reporter (PSE-PBN-LUC). Endogenous AR was activated by the addition of R1881, and AR transcriptional activity was measured relative to control miRNAs. In a second screen, high-throughput Protein Lysate Microarray Technology (LMA) was applied to directly measure the effects of miRNAs on AR protein level and AR activity, through PSA expression. Three AR positive PCa cell lines (LNCaP, VCAP, LAPC4) were transfected with more than 800 miRNA mimetics, followed by protein lysate extraction. Lysates were transferred into 384 well plates using Hamilton Robotics System and arrays were printed on Fast Slide using the GeSiM Nanoplotter2 robotic system. The resulting LMAs were probed for AR, GAPDH and PSA using specific antibodies. Expression intensity was measured and quantified relative to control miRNAs. In a third screen, over 800 miRNA mimetics were transfected into LNCaP cells stably expressing a viability reporter, βActin-Mluc. miRNA influence on PCa cell growth and viability was evaluated, relative to control miRNAs. Candidate AR-targeting miRNAs and targets were characterized by western blotting, 3'UTR reporter assays, and AR reporter assays. miRNAs were also bioinformatically evaluated for association with clinical PCa progression in publically available databases.

Results: Using complementary AR transcriptional assays, proliferation assays, and protein LMAs, we have identified more than 40 miRNAs that suppress the AR signaling axis and proliferation of PCa. Expression of more than 19 of the identified miRNAs was associated with the clinical progression of PCa in a previously published data set. More than 20 miRNAs directly target the long AR 3'UTR (untranslated region), and three miRNAs suppress AR through binding to the coding region. Twelve AR-targeting miRNAs reduce both AR and AR splice-variant 7 (AR-V7) expression levels. Functionally, miR-30 family miRNAs were observed to be more dominant suppressors of proliferation in AR positive cell lines. Two miR-30 family miRNAs (miR-30b and miR-30d) were further selected for testing and were found to inhibit AR expression, AR activity and cellular proliferation in multiple cell lines miR-30b expression was found to be reduced in primary PCa specimens. In addition, both miR-30b and miR-30d suppressed the expression of AR coregulators ATAD2, SMAD3, Cyclin E2, B arrestin, MDM2, indicating the potential for pathway regulation through multiple components of androgen signaling.

Conclusions: A series of complementary and high-throughput assays have identified miRNAs capable of regulating the AR signaling pathway in PCa. We anticipate that expression of some of these miRNAs may be differentially expressed in castration resistant PCa and that these pathways may be exploited for new anti-androgen therapeutics.

P-59

NEW INSIGHTS INTO THE PROCESSING OF PRIMARY tRNA TRANSCRIPTS IN *ESCHERICHIA COLI*

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The *E. coli* genome contains 86 tRNA genes that are organized as either monocistronic transcripts or complex operons containing other tRNAs, messenger RNAs (mRNAs) or ribosomal RNAs (rRNA) genes. Every tRNA is transcribed as a precursor that contains an encoded CCA determinant and requires subsequent processing at both ends to generate mature species that can be charged by their cognate aminoacyl tRNA synthetases. tRNAs that are part of polycistronic transcripts require initial endonucleolytic cleavages to generate the pre-tRNAs that undergo further processing at their 5' and 3' termini. The generally accepted model for such processing has proposed that endonucleolytic cleavages of polycistronic transcripts by RNase E generate pre-tRNAs. Subsequently, the ribozyme RNase P endonucleolytically removes the extra nucleotides at the 5' terminus, while the 3' terminus is processed exonucleolytically by a combination of RNase T, RNase PH, RNase BN/Z, RNase D, RNase II and PNPase. However, we have recently shown that a significant portion of *E. coli* primary transcripts are initially processed by RNase P and not RNase E (1,2). For example, all seven leucine tRNAs (found in three polycistronic transcripts) are initially separated into pre-tRNAs exclusively by RNase P (3). Furthermore, RNase P processes the *valU* (four tRNAs) and *lysT* (seven tRNAs) operons in the 3' → 5' direction by first removing the Rho-independent transcription terminators (3).

We have now also demonstrated that polyadenylation of pre-tRNAs by poly(A) polymerase I exacerbates the conditional lethality associated with mutations in the protein subunit of RNase P, such that inactivation of PAP I leads to partial complementation of the temperature sensitivity associated with the *rnpA49* allele. Perhaps of greater significance, we will present results that demonstrate that short unprocessed 5' regions (1-5 nt) on pre-tRNAs do not interfere with aminoacylation if the 3' ends are fully processed. Finally, we will show that the three primary proline tRNAs are matured in such a fashion that they do not require any 3' → 5' processing by RNase T, RNase PH, RNase BN/Z, RNase D, RNase II or PNPase. This work was supported in part by research grants from the National Institutes of Health to S.R.K. (GM57220 and GM81554).

1. Mohanty, B.K. and Kushner, S.R. (2007) Ribonuclease P processes polycistronic tRNA transcripts in *Escherichia coli* independent of ribonuclease E. *Nucleic Acids Res*, 35, 7614-7625.
2. Mohanty, B.K. and Kushner, S.R. (2008) Rho-independent transcription terminators inhibit RNase P processing of the *secG leuU* and *metT* tRNA polycistronic transcripts in *Escherichia coli*. *Nucleic Acids Res*, 36, 364-375.
3. Agrawal, A., Mohanty, B.K. and Kushner, S.R. (2014) Processing of the seven valine tRNAs in *Escherichia coli* involves novel features of RNase P. *Nucleic Acids Res*, 42, 11166-11179.

P-60

THE BIOLOGICAL IMPACTS OF RIBOSNITCHES: LINKING RNA STRUCTURAL CHANGES WITH MOLECULAR PHENOTYPES

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Single nucleotide variants that affect RNA structure (riboSNitches) were recently discovered through next-generation sequencing structure determination in humans. What phenotypic changes do these riboSNitches cause within a cell? There is evidence that RNA structure is important for both function and regulation. Thus, we hypothesize that riboSNitches can affect RNA stability, translational efficiency and protein binding, and that the majority of riboSNitches will have a functional impact on their transcript. We are currently testing our hypothesis with a multi-pronged approach using genotyped human lymphoblastoid cells. First, we are identifying riboSNitches with allele-specific selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-map) structure determination. Then we are testing RNA stability with a sequencing method based on the classical pulse-chase assessment with bromouridine - 5'-bromo-uridine immunoprecipitation chase-deep sequencing analysis or BRIC-Seq. In addition, we are determining translational efficiency with polysomal fractionation. Finally, we are looking at global changes between RNA folding in lysates versus the test tube with the goal of identifying and testing specific protein binding sites *in vitro*. Done in tandem, in an allele-specific manner, these studies will allow us to determine whether riboSNitches alter the stability, translational efficiency or protein-binding characteristics of their transcripts. Connecting riboSNitches to biological mechanisms is an essential step toward understanding how genotype leads to phenotype and personal variation leads to disease risk.

P-61

INSIGHTS INTO THE FOLDING COOPERATIVITY OF tRNA IN CELLULAR-LIKE CONDITIONS

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The folding of functional RNAs is thought to happen in a hierarchical process with many well-populated intermediates. This type of folding happens over a rugged landscape resulting in many RNAs not folding to the native or functional confirmation in a biological time scale.[1] It has been proposed that the strong secondary structural elements of functional RNAs drives their folding to a native structure.[2] In dilute solution conditions of high magnesium (10 mM or higher), high monovalent salts, and no crowding this rugged folding process has been observed. However, biological conditions are much different from typical solution conditions. It is estimated that in cells there is between 0.5-2 mM Mg2+, 140 mM K+, and 20%-40% macromolecular crowders. Previous work from our lab has shown that under cellular-like conditions RNA folds in a cooperative manner, where intermediates are not highly populated.[3] In cooperative systems the tertiary structure is unfolding simultaneously with the secondary structures. In the work herein the mechanism for RNA folding cooperativity in biological conditions is probed. The folding of tRNAphe and the individual secondary structure elements (hairpins) of tRNAphe are being studied in crowded conditions at biological concentrations of Mg2+ and K+. From comparison of optical melts between tRNAphe and its component hairpins under biological conditions, it is determined that the folding of the full-length tRNAphe becomes two-state and cooperative, while the folding of its isolated secondary structures do not. The cooperative folding under crowded conditions happens via a stabilization of the tertiary structures and a destabilization of the secondary structures. These findings suggest that in conditions that mimic the cell, it is the tertiary contacts that drive folding of many functional RNAs, not the formation of secondary structures.

1. Brion, P. and E. Westhof (1997). "Hierarchy and dynamics of RNA folding." Annu. Rev. Biophys. Biomol. Struct. 26: 113-137.

2. Treiber, D. K.; Rook, M. S.; Zarrinkar, P. P.; Williamson, J. R., Kinetic intermediates trapped by native interactions in RNA folding. Science 1998, 279, 1943-1946.

3. Strulson, C. A., Boyer, J. A., Whitman, E. E. & Bevilacqua, P. C. (2014). Molecular crowders and cosolutes promote folding cooperativity of RNA under physiological ionic conditions. RNA 20, 331-347. PMCID: 3923128.

P-62

A CRITICAL ROLE OF POST-TRANSCRIPTIONAL REGULATION OF NEUROPEPTIDE VGF IN MODULATING COGNITIVE FUNCTION

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Brain expression of VGF (nonacronymic), a secreted neuropeptide precursor, can be induced by hippocampal-dependent learning and physical exercise. VGF-derived peptides have been shown to enhance neuronal activity in the cultured hippocampal neurons, and its direct administration into the hippocampus also promotes neurogenesis and shows antidepressant-like effect in mouse stress model. Studies from germline VGF deficient mice, on the other hand, have revealed a critical role of VGF in memory formation, mood disorder, and energy homeostasis. Regulation of VGF expression on the transcriptional level has been reported previously, which can be mediated through the CREB-dependent gene activation. In this study, we now showed that VGF expression is also controlled by post-transcriptional regulatory mechanism which represses VGF expression. We show evidences from our mouse model that dramatic elevation of VGF protein level both in cultured neurons and in the mouse brains can be observed when this post-transcriptional mechanism is abolished. Phenotypic analysis of this mouse model further showed enhanced memory performance, and anxiolytic and antidepressant-like effects. Our results have therefore revealed a previously unidentified but critical regulatory mechanism in negatively regulating VGF expression in the central nervous system. Extended studies on the mechanistic insights should promise an alternative strategy to modulate endogenous VGF expression with its potential application for the clinical treatment of neurodegenerative and psychiatric disorders.

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ENZYMOLOGY OF PRECURSOR-tRNA PROCESSING: MOLECULAR RECOGNITION OF INHIBITORS, METAL IONS AND SUBSTRATES BY RIBONUCLEASE P

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Ribonuclease P (RNase P) is a divalent metal ion-dependent endonuclease that catalyzes cleavage of the 5' leader of precursor tRNA (pre-tRNA). In most organisms, RNase P is a ribonucleoprotein (RNP) complex consisting of a catalytic RNA (P RNA) and variable numbers of proteins. Recently, protein-only RNase P (PRORP) has been identified in human mitochondria and plants. The human mitochondrial RNase P is composed of three protein subunits: MRPP1, MRPP2 and MRPP3. The MRPP1•MRPP2 subcomplex contains an m^1N^9 tRNA methyltransferase (MRPP1), while MRPP3 belongs to a new class of metallonucleases. The plant PRORP only requires the metallonuclease. The RNP and protein-only forms of RNase P present an excellent model system to compare RNA- and protein-based catalysis (1). In addition, bacterial RNase P is considered an antibacterial target and the human mitochondrial RNase P proteins are indicated in human diseases.

To facilitate rapid characterization of RNase P activity, we developed a real-time fluorescence polarization (FP) assay using a 5' end fluorescently labeled pre-tRNA substrate (2). This FP assay was further optimized for high-throughput screening, from which a natural product derivative, iriginol hexaacetate, was identified as a new mixed inhibitor (Ki = 130 nM, $K$is = 480 nM) of bacterial RNase P.

In RNase P RNA, helix P4 is proposed as the active site. A recent crystal structure proposed that the O4 carbonyl oxygen of the universally conserved bulge (U51) in P4 forms an inner-sphere coordination with a catalytic metal ion (3). To test this hypothesis, we substituted U51 with 4-thiouridine (4SU), 4-deoxyuridine (4deOU), 3-methyluridine (3MU) or a base deletion (abasic, abU). The single-atom substitutions of O4 in U51 (4SU, 4deOU) or removal of the uracil base (abU) decrease the single-turnover cleavage rate constant by 20-fold and have little effect on the rate constant of a conformational change step that occurs before the cleavage step in Mg^{2+}. Interestingly, a 3- to 5-fold rescue of activity of the 4SU substitution is observed upon addition of thiophilic metal ions, Cd^{2+} or Mn^{2+}, consistent with inner-sphere metal ion coordination. In addition, the 3MU substitution retains a wild-type-like cleavage rate constant but the rate constant for the conformational change decreases by 28-fold. This work provides important biochemical evidence of inner-sphere coordination of a divalent metal ion with a nucleotide base in P RNA as well as strong evidence demonstrating that the conformational change in the catalytic pathway of RNase P occurs in or near the P4 helix.

We have also purified and characterized the *in vitro* activity of the human mitochondrial RNase P subunits. Single-turnover cleavage data show that MRPP3 alone catalyzes the removal of the 5' leader of a mitochondrial pre-tRNA substrate, albeit slowly and with substantial miscleavage. Addition of the MRPP1•MRPP2 subcomplex significantly increases the cleavage rate constant (1000-fold) and substrate affinity (50-fold) and diminishes miscleavage. In addition, *in vitro* pull-down results indicate that MRPP3 has highest affinity for the (MRPP1•MRPP2)•pre-tRNA complex. These data suggest that MRPP3 recognizes the MRPP1•MRPP2-bound pre-tRNA as substrate. This unique strategy for substrate recognition may be evolved specifically for the non-canonical features of human mitochondrial tRNAs (4). [Funding from NIH (GM 55387) supports this work].

1. Howard, M. J., Liu, X., Lim, W. H., Klemm, B. P., Fierke, C. A., Koutmos, M., and Engelke, D. R. (2013) RNase P enzymes: divergent scaffolds for a conserved biological reaction. *RNA Biol* 10, 909-914
2. Liu, X., Chen, Y., and Fierke, C. A. (2014) A real-time fluorescence polarization activity assay to screen for inhibitors of bacterial ribonuclease P. *Nucleic Acids Res* 42, e159
3. Reiter, N. J., Osterman, A., Torres-Larios, A., Swinger, K. K., Pan, T., and Mondragon, A. (2010) Structure of a bacterial ribonuclease P holoenzyme in complex with tRNA. *Nature* 468, 784-789
4. Suzuki, T., Nagao, A., and Suzuki, T. (2011) Human mitochondrial tRNAs: biogenesis, function, structural aspects, and diseases. *Annu Rev Genet* 45, 299-329

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SMG1 FUNCTIONS IN THE NONSENSE-MEDIATED mRNA DECAY PATHWAY OF PLANTS AND IS IMPORTANT FOR NORMAL GROWTH

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The regulation of gene expression is not simply confined to the activity of a promoter but can occur at many stages, including mRNA degradation. Nonsense-mediated mRNA decay (NMD) is a eukaryotic mRNA decay pathway. It was first characterised as a pathway degrading transcripts with premature stop codons arising from mutations. However, NMD also directly targets many 'non-aberrant' transcripts and is important for normal growth and development. For example, NMD is needed for a normal response to pathogens in *Arabidopsis thaliana* and NMD is regulated during mammalian brain development. In animals, it is well known that the kinase SMG1 activates the NMD pathway when a premature stop codon is recognised but no NMD-associated kinase has been characterised outside the animal kingdom. Here we report that *SMG1*, whilst missing from fungi and *A. thaliana*, is ubiquitous in the plant kingdom, functions in the NMD pathway of moss and is needed for normal moss development. An RNA-seq analysis of transcripts regulated by SMG1 in moss revealed that NMD is important for regulating the unfolded protein response and is also involved in the DNA repair pathway. Taken together, we have shown that SMG1 is an ancient kinase, which functions in the NMD pathway in moss. Furthermore, NMD is important for normal moss development.

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HEPATITIS C VIRUS RNA FUNCTIONALLY SEQUESTERS miR-122

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Hepatitis C virus uniquely requires the liver specific microRNA-122 for replication, yet global effects on endogenous miRNA targets during infection are unexplored. Here, high-throughput sequencing and crosslinking immunoprecipitation (HITS-CLIP) experiments of human Argonaute (Ago) during HCV infection showed robust Ago binding on the HCV 5'UTR, at known and predicted miR-122 sites. On the human transcriptome, we observed reduced Ago binding and functional mRNA de-repression of miR-122 targets during virus infection. This miR-122 "sponge" effect was relieved and redirected to miR-15 targets by swapping the miRNA tropism of the virus. Single-cell expression data from reporters containing miR-122 sites showed significant de-repression during HCV infection depending on reporter expression level and number of miRNA sites. Furthermore, sponge effects could be observed in cells with *in vivo*-like levels of miR-122. We describe a quantitative mathematical model of HCV induced miR-122 sequestration and propose that such miR-122 inhibition by HCV RNA may result in global de-repression of host miR-122 targets, providing an environment fertile for the long-term oncogenic potential of HCV.

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REGULATION OF COX-2 EXPRESSION BY miRNAS AND CYTOKINES IN LUNG CANCER CELLS

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The oxidative conversion of arachidonic acid to prostaglandin H_2 is carried out by a set of two enzymes termed cycloxygenases, abbreviated as COX. COX-1 is constitutively expressed in normal tissues, while COX-2, which is not expressed in normal tissues, is transiently induced from external stimuli, such as pro-inflammatory cytokines. COX-2 is also overexpressed in numerous cancers. We show that COX-2 protein expression is constitutive in several lung cancer cell lines, but not expressed in a normal bronchial cell line, Beas2B. Previous work from our lab has shown that COX-2 expression can be regulated by alternative polyadenylation through usage of alternative poly(A) sites. Another means of post-transcriptional regulation is mediated through microRNA repression. We have Real-Time qPCR data and microarray data that show decreased expression of specific miRs in lung cancer cells as compared to normal lung cells. The biological function of COX-2 is to produce prostaglandins; we have also demonstrated that COX-2 specific miRs can modulate resulting prostaglandins produced. In addition, cytokines can regulate miR and COX-2 expression. We speculate that many post-transcriptional mechanisms work in concert in our system.

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THE SUPPRESSIVE ACTIVITY OF SRSF3 ON ALTERNATIVE SPLICING IS ATTENUABLE DURING KSHV INFECTION BY VIRAL POSTTRANSCRIPTIONAL REGULATOR ORF57

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SRSF3 (formerly known as SRp20) is the smallest member of the SR protein family that regulates alternative splicing by interacting with RNA cis-elements in a concentration- and cell differentiation-dependent manner. We recently demonstrated that SRSF3 is a host modulator of gene expression of oncogenic Kaposi sarcoma-associated herpesvirus (KSHV) with suppressive activity towards alternative splicing of a viral K8 transcript. By interacting with multiple regions in a suboptimal intron (intron 2 or K8beta intron), SRSF3 inhibits K8 splicing resulting in the K8beta intron inclucion which contains a premature stop codon and thus the expression of a truncated beta form of K8 protein. The specific knock-down of SRSF3, but not other SR proteins, activates splicing of the K8beta intron and expression of a functional full length alpha form of K8 protein essential for replication of viral genome. During KSHV productive infection, the suppressive activity of SRSF3 is attenuated by viral ORF57, a multifunctional posttranscriptional regulator, that directly interacts with the SRSF3 N-terminal RNA-recognition motif (RRM). ORF57 binding to SRSF3 RRM interferes with its RNA-binding potential, prevents SRSF3 association with the K8beta intron, and thus promotes efficient splicing of the K8beta intron and expression of functional K8 protein. Interestingly, the SRSF3 activity on K8beta splicing could be regulated by members of cellular SPEN protein family RBM15 and OTT3. RBM15 competes with SRSF3 for ORF57 binding in dose-dependent manner and thus disrupts the interaction of ORF57 and SRSF3. This observation confirms a proposed mechanism of attenuation of suppressive activity SRSF3 on alternative splicing as a result of direct protein-protein interaction of SRSF3 with KSHV ORF57 protein. Moreover, ORF57 also affects SRSF3-dependend splicing events of other non-KSHV transcripts and has general effects on SRSF3 activities.

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ROLE OF hPNPase IN REGULATING OXIDIZED RNA

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hPNPase is a human homologue of polynucleotide phosphorylase (PNPase) that belongs to the PDX family of phosphorolytic exoribonucleases that digest RNA in the 3'→5' direction. PNPase enzymes usually exhibits multiple biochemical activities including RNA degradation, synthesis of RNA, RNA binding, etc. hPNPase was found predominantly in the mitochondrial intermembrane space (IMS), however, transient localization in the mitochondrial matrix or cytosol was also proposed. hPNPase carries out a number of functions inside the cell such as gene regulation, maintenance of mitochondrial homeostasis and morphogenesis, and regulation of respiratory chain components. hPNPase has been shown to function in multiple aspects of RNA metabolism in including mitochondrial RNA processing/import and degradation of mitochondrial and cytosolic mRNAs and microRNAs. More recently, hPNPase has been shown to play an important role in reducing oxidatively damaged RNA. It binds to RNA made of an oxidized form of guanine, 8-hydroxyguanine (8-oxoG), with high affinity. Overexpression of hPNPase in HeLa reduces 8-oxoG levels in cellular RNA. Moreover, HeLa cells lacking hPNPase contain elevated levels of 8-oxoG in RNA and become hypersensitive to oxidative stress. RNA oxidation is strongly implicated in a number of human diseases, suggesting that hPNPase may play important roles in preventing the diseases through RNA quality control. In this study, we have elucidated the mechanisms by which hPNPase reduces 8-oxoG in cellular RNA. Also, it has been shown that hPNPase controls 8-oxoG in the cytosol and mitochondria. In mitochondria, hPNPase forms a stable complex with an RNA helicase hSUV3, and interact with the mitochondrial poly (A) polymerase (mtPAP). Both the latter enzymes are known to facilitate RNA degradation. Interestingly, we also showed that hSUV3 and mtPAP play a very important role in reducing oxidized RNA inside mitochondria. Therefore, hPNPase including mtPAP and hSUV3 could be very instrumental in regulating RNA oxidation and associated human diseases.

P-69

HYPOXIA AND HYPOGLYCEMIA SYNERGISTICALLY REGULATE mRNA STABILITY

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Rapidly growing tumors are often poorly vascularized, resulting in impaired delivery of oxygen and glucose to the cells that make up the tumor. The cellular response to this nutrient deprivation will often determine the severity of the cancer and the ultimate prognosis for the patient, and as such represents an important therapeutic target. While much is known about the cellular transcriptional response to hypoxia (mediated primarily through the Hypoxia Inducible Factor pathway), much less is known about the posttranscriptional response to oxygen and glucose deprivation. The goal of this study is to characterize one such posttranscriptional response, namely the regulation of mRNA stability, in response to oxygen and glucose deprivation.

We have identified a number of ischemia-related mRNAs that are synergistically stabilized by oxygen and glucose deprivation including VEGF, C-MYC and CYR61. This increase in mRNA half-life requires the synergistic effects of both low oxygen (1%) as well as low glucose (1 g/L) conditions. Oxygen or glucose deprivation alone fails to initiate the response, as exposure to either high glucose (4 g/L) or normoxic conditions inhibits the response. The mRNA stabilization appears to be prolyl hydroxylase and HIF independent as hypoxic mimetics Deferoxamine (DFX) or Cobalt Chloride are unable to substitute for the oxygen deprivation. Instead, the stability of these mRNAs appears to be regulated by the RNA binding protein KHSRP, following cellular signaling events that require both functional mitochondria and the P38 MAP kinase pathway. Work is ongoing to fully elucidate the pathways and players involved in this response and to identify the global subset of mRNAs affected.

Through this work, we aim to identify novel players in the ischemic response that can be exploited as therapeutic targets to alter the cellular response to an ischemic event, which in turn may improve a patient's prognosis and recovery from diseases such as cancer.

This work was supported by startup funds from East Carolina University.

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THE MAMMALIAN La-RELATED PROTEIN 4 mRNA CONTAINS A FUNCTIONAL AU-RICH ELEMENT AND ITS PROTEIN LEVELS ARE REGULATED BY TRISTETRAPROLIN

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La-related protein 4 (LARP4) is a newly described RNA binding protein that is involved in general translation. To learn more about its function, we are generating a LARP4 KO mouse, and are also examining its regulation, the topic of today's talk. The online ARED organism search engine predicts a possible AU-rich element (ARE) in the 3'-UTR of the LARP4 mRNA. AREs are mammalian cis-acting sequences of 50-150 nt within the 3'-UTRs of 5-8% of human mRNAs. These mRNAs mostly encode proteins that need to be tightly regulated, e.g. transcription factors, cytokines and cell-cycle genes. It was reported that in colorectal cancer (CRC), ARE-containing genes are enriched in expression. Interestingly, LARP4 is also found to be up regulated in CRC.

To assess if the newly discovered ARE in LARP4 mRNA may be functional, we adopted a Tet-off system to monitor the stability of a specific mRNA reporter under a dox-responsive promoter and derivatives in HeLa Tet-Off cells. Adding doxycycline shuts off the dox-responsive promoter without affecting endogenous mRNA transcription. Inserting the putative LARP4 ARE in the 3'-UTR of the mRNA reporter decreased the half-life from over 6 hours to 100 minutes. Point mutations to the ARE rescued the stability of the reporter mRNA. By performing RNA co-immunoprecipitation, we show that the ARE-binding protein, Tristetraprolin (TTP), binds to LARP4 mRNA *in vivo*. Over-expression of wild-type TTP, but not its RNA binding mutant or other ARE-binding proteins tested, decreases LARP4 protein levels but surprisingly without affecting LARP4 mRNA levels. This is very exciting since not much is known about the mechanism by which ARE binding proteins can affect the levels of proteins derived from the ARE-containing mRNAs. Consistent with this, mouse LARP4 protein levels are higher in a TTP knock out cell line vs. a wild type control. When these cells are stimulated with TNFa to induce TTP, LARP4 protein levels are down regulated only in the wild type cell line. So LARP4 is down regulated in a TTP dependent manner. Currently we are investigating if induction of TTP displaces LARP4 mRNA from actively translating ribosomes and thus inhibits its translation.

In conclusion, we are the first to show that the expression of a La-related protein is regulated by an ARE in the 3'-UTR. And, more importantly, that this happens by decreasing the protein level instead of enhancing mRNA decay.

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LIPOSOMAL DELIVERY OF A microRNA-145 INHIBITOR IMPROVES LUNG STRUCTURE AND HEART FUNCTION IN EXPERIMENTAL PULMONARY HYPERTENSION

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Rationale. Therapies which exploit RNA interference (RNAi) hold great potential for improving disease outcomes. However there are several challenges which limit the application of RNAi therapeutics related primarily to effective delivery of oligonucleotides to target cells and reduced delivery to non-target cells. We have previously developed a functionalized cationic lipopolyamine (Star:Star-mPEG) for *in vivo* delivery of siRNA to pulmonary vascular cells. This optimized lipid formulation enhances the retention of siRNA in mouse lungs and achieves significant knockdown of target gene expression for at least 10 days following a single intravenous injection. Although this suggests a strong potential for developing lung directed RNAi based therapies, the application of Star:Star-mPEG mediated delivery of RNAi based therapies for pulmonary vascular diseases such as pulmonary arterial hypertension (PAH) remains unknown. We identified differential expression of several microRNAs known to regulate cell proliferation, cell survival and cell fate that are associated with development of PAH, including increased expression of microRNA-145 (miR-145).

Approach. Here we test the hypothesis that Star:Star-mPEG mediated delivery of an antisense oligonucleotide against miR-145 (antimiR-145) will improve established PAH in rats. We performed a series of experiments testing the *in vivo* distribution, toxicity, and efficacy of Star:Star-mPEG mediated delivery of antimiR-145 in rats with Sugen-5416/Hypoxia induced PAH.

Results. We show that after subchronic therapy of three injections over 6 weeks at 2 mg/kg, antimiR-145 accumulates in rat lung tissue and reduces expression of endogenous miR-145. Using an in situ hybridization approach, we demonstrate substantial distribution of antimiR-145 in lungs as well as liver, kidney, and spleen. We assessed toxic effects of Star:Star-mPEG/antimiR-145 with serial complete blood counts of leukocytes and serum metabolic panels, gross pathology, and histopathology. We detected no change in mean body weight or spleen weight, and no evidence of renal, hepatic or hematopoietic toxicity after 6 weeks of therapy. We measured the degree of pulmonary arteriopathy, the severity of pulmonary hypertension, and the degree of cardiac dysfunction. The results demonstrate that Star:Star-mPEG delivery of antimiR-145 inhibitor elicits therapeutic effects in experimental, severe PAH. Lung delivery of antimiR-145 reduced wall thickness in arteries ranging in size from 50-200 μm and reduced the density of occlusive vascular lesions. The anti-remodeling effects of antimiR-145 were associated with repair of heart structure and improved cardiac function. Although the dosing schedule has not been fully optimized, these results suggest a low frequency (biweekly), low dosing (2 mg/kg) approach is sufficient to achieve significant pulmonary vascular repair that improves cardiac function.

Conclusion. These results establish effective lung delivery of a miRNA-145 inhibitor using a functionalized cationic lipopolyamine to repair pulmonary arteriopathy, reduce established pulmonary hypertension, and improve cardiac function in rats with severe PAH without adverse effects. Lung delivery, low toxicity, and robust efficacy make this formulation appealing for development as an RNAi therapy to reverse the arteriopathy and RV failure of PAH to improve patient quality of life and survival.

P-72

THE 1213-NT FISSION YEAST TELOMERASE RNA SUBUNIT TER1 IS A FLEXIBLE SCAFFOLD

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Telomeres are repeating DNA sequences and their associated proteins found at the ends of chromosomes, which are elongated by the enzyme telomerase. The core enzyme of telomerase consists of a reverse transcriptase and a long non-coding RNA subunit. These two components can reconstitute telomerase activity *in vitro*, whereas *in vivo* several accessory proteins are also required. The majority of adult human somatic cells have essentially completely repressed telomerase expression so their telomeres are shortened with every round of replication. When telomeres become critically short cells can no longer continue to divide and, therefore, telomeres ensure a limited replicative lifespan. Conversely, 85–97% of cancer cells, which are immortalized, exhibit aberrant telomerase upregulation. Previous research on *Saccharomyces cerevisiae* telomerase RNA, TLC1, has led to a functional miniaturized allele, indicating that two-thirds of this 1157-nt RNA is dispensable for function. TLC1 also acts as a flexible scaffold for holoenzyme protein subunits; the Est1, Ku and Sm_7- -binding regions can be repositioned on the RNA with retention of function. The fission yeast *Schizosaccharomyces pombe* is evolutionarily distant from *S. cerevisiae* yet its 1213-nt telomerase RNA, TER1, is similar in size to TLC1. Unlike TLC1, TER1 shares with the human telomerase RNA the requirement for a three-way junction domain for catalytic activity. While several regions of the TER1 structure have been studied and modeled, a complete, well-tested secondary structure model of the RNA does not yet exist. Phylogenetic analysis of TER1 is difficult because telomerase RNAs are evolving very rapidly and only four species of fission yeast have been identified. Therefore, alternative approaches are necessary in order to develop and test a complete model of the RNA. Using an *in vitro* reconstituted activity assay, we have created an active 623-nt Micro-TER1 RNA that contains the catalytic core as well as the essential three-way junction region. We are now working on testing a smaller Micro-TER1 to define the minimal amount of RNA required for catalytic activity. We have also used truncation mutants in genetic complementation tests to determine which regions of the RNA are essential *in vivo*, we find that about 40% of the RNA is dispensable. *In vivo* tests have also shown that the essential three-way junction region can be relocated, providing evidence that TER1, like TLC1, is acting as a flexible scaffold. Lastly, using base-pair compensatory mutants, we are testing models for the TER1 pseudoknot, a conserved motif in the catalytic core of telomerase RNAs.

P-73

RELOCATING THE ENDS OF HUMAN TELOMERASE RNA TO NEW POSITIONS REVEALS INSIGHTS INTO RNP ARCHITECTURE AND MECHANISM

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Telomeres are repetitive sequences at the ends of linear eukaryotic chromosomes. Owing to the nature of DNA polymerases, telomeres cannot be complete replicated due to the end-replication problem. Most eukaryotes utilize the ribonucleoprotein complex telomerase to maintain telomere length. Telomerase is minimally comprised of a telomerase RNA, which provides a template, and a reverse transcriptase protein (TERT), which catalyzes the addition of nucleotides to the 3' end. Most human somatic cells do not express telomerase; however, telomerase is up-regulated in more than 85% of cancers and seems to be required for the unlimited proliferative capability of cancer cells. To better understand the function of human telomerase RNA (hTR) we have tested 46 circular permutations throughout the full-length 451-nt hTR using an *in vitro* activity assay. Circular permutations reposition the location of the 5' and 3' ends, essentially introducing a nick in the phosphate backbone. Our results reveal several important areas of connectivity within hTR. First, we find that circular permutations 3' of the template have defects in repeat addition processivity. This suggests functional similarity with the analogous template recognition element in *T. thermophila* telomerase RNA even though these RNAs are evolutionarily distinct. Second, we find that circular permutations in the G-rich 5' end and between the conserved core and the CR4/5 region increase telomerase activity. Third, several circular permutations in and around the base triples within the pseudoknot or the P6.1 helix in the CR4/5 region completely abolish telomerase activity. Together, this comprehensive exploration of RNA connectivity requirements in hTR extend our current understanding of telomerase RNA function. Further, the last class of mutants represent attractive candidates for developing anti-cancer therapeutics.

P-74

THE EFFECT OF THE 7-METHYLGUANOSINE CAP ON THE STRUCTURE OF THE HIV-1 5'-LEADER

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HIV remains a serious global health concern with 1.5 million deaths due to HIV in 2013 alone and approximately 35 million people currently living with HIV. Despite the success of rational drug design in developing anti-retrovirals, drug resistance is an increasing problem, creating a demand for new therapies. An area of interest for drug development is the 5'-leader (5'-L), which is the most highly conserved region of the RNA genome and controls a number of important processes in the HIV life cycle. Research has shown that the 5'-L acts as a structural switch, existing in a monomeric conformation, which controls splicing and translation of the genome, and a dimeric conformation, which is selectively packaged into new viral particles. Investigations of the structure of the monomeric conformation of the 5'-L have revealed that the presence of the 5'-7-methylguanosine cap (5'-cap) on the 5'-L stabilizes the monomeric conformation. A structural role for the 5'-cap is unanticipated because it is expected to be sequestered by binding of the cap-binding protein. Additional studies suggest that the base pairing of residues at the bottom of the predicted PolyA hairpin influence the monomer-dimer equilibrium. Mutations that stabilize the bottom of the PolyA hairpin stabilize the dimeric conformation. Conversely, mutations that destabilize the bottom of the PolyA hairpin stabilize the monomeric conformation. Together these data support a theory that the 5'-cap may interact with residues previously predicted to be part of the PolyA hairpin to influence the monomer-dimer equilibrium. Nuclear magnetic resonance studies will be used to elucidate the interactions of the 5'-cap with the 5'-L.

P-75

DEVELOPMENT OF AN RNA-TARGETED SMALL MOLECULE LIBRARY FOR THE DISRUPTION OF THE LONG NON-CODING RNA-PROTEIN INTERACTIONS

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Long non-coding RNAs (lncRNAs) play a role in many important cellular processes and have altered expression in various diseases. One example, HOX antisense intergenic RNA (*HOTAIR*), is overexpressed in several metastatic cancers, and overexpression in cell culture and mouse models leads to a significant increase in metastatic tumors. While multiple protein-binding partners for *HOTAIR* have been identified, the molecular interactions responsible for the altered phenotype are largely unknown because current tools are limited in their ability to probe individual lncRNA-protein interactions. We propose that multivalent small molecule inhibitors will offer sufficiently high specificity and affinity for RNA to probe specific lncRNA-protein interactions. Despite recent advances in RNA-small molecule targeting, however, there is a significant need to improve the quality of RNA-targeted libraries by further understanding how small molecules recognize RNA. Therefore, we are designing and synthesizing a small molecule library based upon known RNA-privileged scaffolds. Toward this goal, we herein describe the synthesis of oxazolidinone small molecules, which have been shown to recognize both ribosomal RNA and the T-box riboswitch. Currently, two synthetic routes are being explored toward scaffolds with the ability to be diversified at the N3, C4, and C5 positions. One synthesis begins with a mono-protected 3-butene-1,2-diol. After conversion to an azidoformate, iron-catalyzed cyclization yields a scaffold with three orthogonal functional groups for diversification. The second synthesis incorporates Boc-protected chiral amino acid methyl esters that are cyclized via a brominated intermediate to form a tri-substitutable scaffold. The scaffold is diversified at the N3 position by alkylation or arylation, the C4 position by reaction with primary amines, and the C5 position is defined by the identify of the amino acid. The diverse oxazolidinone library will be combined with other RNA-targeted small molecules, screened for disruption of specific lncRNA-protein interactions, and multiple small molecule hits will be combined into a single ligand by dynamic combinatorial chemistry. The multivalent inhibitors of specific lncRNA-protein interactions will be evaluated *in vitro* and cell culture to better understand the molecular interactions of lncRNAs in disease.

P-76

MUTATION OF A CNS-SPECIFIC tRNA INDUCES NEURODEGENERATION

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Transfer RNA (tRNA) genes are critical adaptor molecules for protein translation and hence their genetic architecture must ensure their robust and accurate function. To this end, tRNA genes are typically multicopy, i.e. an anticodon is represented by several (sometimes several dozen) tRNA genes, which are usually scattered across the genome. Importantly in higher organisms, these tRNA copies often are not identical but are isodecoders, i.e., tRNA genes that share an anticodon but differ in their body sequence. Traditionally, individual isodecoders have not been attributed unique characteristics, but were assumed to be functionally interchangeable within an isodecoder family. Interestingly, we have recently identified a mouse tRNA (and its human orthologue) that is expressed in a highly tissue-specific manner.

Mice homozygous for the ENU-induced *nmf205* mutation, which abolishes expression of GTPBP2, a novel rescue factor for stalled elongation complexes, have progressive neurodegeneration which begins at 1 month of age. Although *Gtpbp2* is widely expressed, cell death is strikingly confined to the CNS. We observed that genetic backgrounds other than C57BL/6J greatly attenuated the *nmf205*-associated phenotype. Using genetic mapping and transgenic rescue experiments, we demonstrated that enhancement of the *nmf205* phenotype on a C57BL/6J background is due to a processing mutation in a tRNAArg. Intriguingly, we found that this tRNAArg is unique as it is expressed exclusively in the CNS, whereas its isodecoders are widely expressed. Ribosome foot-printing experiments demonstrated that loss of this tRNA, which makes up approximately two-thirds of its isodecoder pool, leads to ribosome stalling in the brain that is rescued by GTPBP2. Together our studies have identified the first tissue-specific tRNA in mammals and pinpointed ribosome stalling as a novel mechanism of neurodegeneration.

P-77

ROLE OF PKR AND RISC-LOADING COMPLEX IN METABOLIC DISEASE

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Aberrant inflammatory responses and protein translational control are key factors in the pathogenesis of metabolic dysfunction in obesity. We recently showed that double-stranded RNA-dependent protein kinase (PKR), an established pathogen sensing protein, is involved in the critical aspects of metabolic dysfunction.

To further investigate the impact of PKR on metabolism and the underlying mechanisms, we generated genetically obese mice (*ob/ob*) with PKR deficiency. In this setting, body weight was not affected by the absence of PKR, yet PKR-deficiency still resulted in significantly improved insulin sensitivity along with reduced JNK activation and eIF2a phosphorylation. Similarly, treatment of *ob/ob* mice with two structurally distinct small-molecule inhibitors of PKR reduced adipose tissue inflammation, improved insulin sensitivity, and improved glucose intolerance.

More recently, through mass spectrometry analyses, followed by biochemical and molecular validation experiments, we determined that PKR forms complexes with components of RISC (RNA-induced silencing complex)-loading complex, which plays a central role in generating microRNAs. These complexes preferentially assemble when PKR is activated by inflammation, excess nutrients, and in the obese but not lean liver. The interactions between PKR and components of RISC require PKR's RNA-binding domains, which are essential for PKR activation in these conditions. In addition, disruption of the PKR-RISC-loading complex results in markedly diminished JNK activation and eIF2a phosphorylation, and a drastic improvement of obesity-induced systemic insulin resistance and glucose metabolism. Our data demonstrate that PKR plays a central role in coordinating multiple networks to integrate metabolism with stress signals through formation of a metabolic inflammatory complex, a metaflammasome, which contains RNA-binding proteins, and RNA species in metabolic diseases.

P-78

METFORMIN AND LIFESPAN EXTENSION: THE ROLE OF DICER AND microRNAs

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Metformin has been commonly used for decades to treat type 2 diabetes. Recent data indicates that mice treated with metformin live longer and healthier lives. Here, we show that chronic metformin exposure in mice increases expression of the microRNA (miRNA) processing protein, Dicer. In agreement with this finding, we found differential changes in miRNA expression in mice treated with metformin or caloric restriction, a proven life extending intervention. Several of these miRNAs are important for regulating cellular senescence and lifespan in model organisms. Consistent with this observation, doses of metformin in the therapeutic range decreased cellular senescence, an *in vitro* model of cellular aging. Genome-wide analysis of gene and miRNA expression changes identified miRNA target networks that are important for mediating the effects of metformin or caloric restriction. In humans, we found that *DICER1* mRNA levels decrease with age. These data lead us to hypothesize that changes in Dicer levels may be important for organismal aging and that interventions that upregulate Dicer expression (e.g., metformin) may offer new therapeutic approaches to combat or prevent age-related diseases.

P-79

REPRESENTATION OF NON-CODING RNAs IN THE MAMMALIAN REFERENCE SEQUENCE DATABASE

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The Reference Sequence (RefSeq) project (www.ncbi.nlm.nih.gov/refseq/) at the NCBI provides a database of annotated sequence standards for transcripts, proteins and genomes. Among the many transcript types represented in the RefSeq collection are the non-coding RNAs (ncRNAs) which include small ncRNAs, ribosomal RNAs, transcribed pseudogenes and long non-coding RNAs (lncRNAs). RefSeq currently represents over 360,000 records of mammalian ncRNAs (NR_ and XR_ accession prefixes) including approximately 66,000 in human and mouse. Small non-coding RNAs are defined into specific classes based on homology and functional conservation among model organisms. Our annotations of these small RNAs are based on a combination of in-house computational predictions and data imported from outside collaborators such as miRBase. In contrast, RefSeqs for transcribed pseudogenes and lncRNAs are mostly generated through NCBI's eukaryotic computational genomic annotation pipeline supplemented by manual sequence analysis and curation by NCBI scientific staff. Transcribed pseudogene loci are defined by non-protein-coding transcripts with homology to a parental protein-coding gene. lncRNAs encompass a more disparate set of genes, including splice variants of protein-coding genes, and present a unique curatorial challenge because they often lack cross-species homology and can be weakly expressed. A small but growing number of lncRNAs have been the subject of experimental investigation, and many of these have been associated with various cancers (e.g. HOTAIR, XIST and MALAT1). The proliferation of next generation sequencing data has allowed for the discovery of many previously undetected lncRNAs. Recently we have incorporated this high throughput data into our automatic and manual curation processes and this has resulted in a significant expansion of our representation of this intriguing new class of genes.

P-80

REGULATION OF MYOGENESIS BY MYF5 RNA-BINDING ACTIVITY

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Background. Skeletal muscle contains long multinucleated and contractile structures known as myotubes, generated from the fusion of myoblasts arising from satellite cells. Myogenic regulatory factors (MRFs) are master regulators of skeletal myogenesis. MYF5 is the earliest MRF expressed during myogenesis and functions as a transcription factor in muscle progenitor cells and myocytes. In mouse C2C12 myocytes, MYF5 shows a cyclic expression pattern during the cell cycle and is rapidly reduced in myoblasts committed to differentiation. Although MYF5-null mice do not show significant differences in satellite cell numbers, they display impaired muscle regeneration associated with increased muscle fiber hypertrophy and delayed differentiation. These findings indicate that MYF5 is essential for muscle regeneration after injury despite the maintained transcriptional activity of MYOD, which normally compensates for the absence of MYF5. Thus, we hypothesized that MYF5, in addition to its transcriptional activity, may also play posttranscriptionally regulate muscle-specific gene expression and thereby modulate myogenesis.

Results and Conclusions. In keeping with earlier reports, MYF5 was highly expressed in growing mouse C2C12 myoblasts and declined during differentiation, supporting the involvement of MYF5 in early myogenesis. RNP immunoprecipitation (RIP) analysis showed that MYF5 bound a subset of mRNAs; prominently among them was *Ccnd1* mRNA, which encodes the key cell cycle regulator CCND1 (Cyclin D1). We found that MYF5 was capable of binding the 3' untranslated region (UTR) and the coding region (CR) of *Ccnd1* mRNA and promoted its translation. In addition, MYF5 modestly altered the transcription of *Ccnd1* mRNA in growing myoblasts. In skeletal muscle from young and old mice, MYF5 levels were slightly upregulated with age; CCND1 levels were robustly elevated in muscle from old mice, without a corresponding increase in *Ccnd1* mRNA, further supporting the translational regulation of CCND1 levels and suggesting that MYF5 might regulate this process. Importantly, silencing MYF5 reduced myoblast growth as well as differentiation of myoblasts into myotubes, while overexpressing MYF5 in C2C12 cells upregulated CCND1 expression. We propose that through binding to *Ccnd1* mRNA and promoting CCND1 translation, MYF5 enhances early myogenesis.

P-81

MULTIPLE MECHANISTIC FEATURES OF RNAPII TERMINATION BY 5′-3′ EXORIBONUCLEASE RAT1

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In *Saccharomyces cerevisiae*, there are at least two distinct pathways for RNA polymerase II (RNAPII) termination. One pathway involves Nrd1/Nab3/Sen1 complex to terminate non-coding RNA transcription and is independent of cleavage of nascent RNA transcript. In contrast, the other termination pathway for most protein-coding genes requires cleavage of nascent transcripts by cleavage/polyadenylation factors, and RNA degradation from the newly formed 5' phosphorylated end by 5′- 3′ exoribonuclease Rat1.

Rat1 is known to promote termination of RNAPII on protein-coding genes in complex with Rai1, but its underlying molecular mechanism is still poorly understood. Using *in vitro* transcription termination assays, we have found that RNAPII is prone to terminate more effectively by Rat1/Rai1 when its catalytic site is disrupted due to NTP misincorporation, proposing that paused RNAPII often found *in vivo* near termination sites might adopt similar configuration for Rat1/Rai1 to trigger termination. Intriguingly, yeast Rat1/Rai1 does not terminate *E. coli* RNAP, implying that specific interaction between Rat1/Rai1 and RNAPII may be required for termination. Furthermore, the efficiency of termination increases as the RNA transcript being degraded by Rat1 gets longer, which suggests that Rat1 may generate a driving force to dissociate RNAPII from the template while degrading the nascent transcripts. These results indicate that multiple mechanistic features contribute to Rat1-mediated termination of RNAPII.

P-82

NOVEL CARRIER FOR EFFICIENT DELIVERY OF FUNCTIONAL RNA NANOPARTICLES

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RNA nanotechnology offers several advantages in the formulation of RNAi-based therapeutics, such as control over the composition, programmability, and size/shape/charge aspects of functional RNA nanoparticles. However, the efficient delivery of RNA nanoparticles to cancer cells is a challenging task due to RNA's chemical instability, negative charge, and inability to effectively cross biological membranes. Therefore, various polycationic carriers, such as lipid-based assemblies, are being developed and tested for non-viral delivery of therapeutic RNAs. However, the use of such assemblies often prevents the function of the targeting moieties, such as RNA or DNA aptamers, and thus, such assemblies are only suitable for passive targeting. Here we investigate the complexation between the novel transfecting agent βCD-P-(HMA$_{75}$-*co*-DMAEMA$_{34.3}$-*co*-TMAEMA$_{38.7}$)$_{5.5}$ ("Smart βCD polymer") and various RNA nanoparticles functionalized with siRNAs and aptamers for specific delivery to cancer cells. The interaction is mediated by the electrostatic interactions of the positive quaternary amine groups in the grafts attached to the β-cyclodextrin core and the negative phosphate groups on the RNA nanoparticles. The results show the dependence of the delivery efficiency on composition, size, and shape of RNA-based nanoparticles.

P-83

VIRTUAL SCREENING AIDED DESIGN, SYNTHESIS AND SAR STUDY ON AMILORIDE DERIVATIVES AS PROBES FOR HIV-1-*TAR* RNA

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Design of small molecule probes for RNA is particularly challenging. The structure-guided approaches employed for ligand design against protein targets, for example, are largely unsuccessful in providing useful small molecule probes for RNA. We are currently pursuing the development of an RNA-targeted small molecule library, and our initial efforts have involved the use of computational docking to an ensemble of HIV-1-*TAR* RNA structures as a model system. We designed a series of amiloride derivatives based on the selectivity of dimethyl amiloride (DMA) for the *TAR* apical loop. Based on the docking results, twenty-five analogs representing maximum possible variation in structure and selectivity of docking position were selected for synthesis. We have developed general synthetic routes for rapid, parallel synthesis of a series of diversified amiloride analogs by varying the substituents on two positions of the core pyrazine structure. These analogs were then evaluated for binding using a fluorescent *TAR*-Tat displacement assay and isothermal calorimetry (ITC). These initial efforts have resulted in ligands with greater than 10-fold improved activity relative to DMA. The binding mode of these compounds to *TAR* was then evaluated using the [^{13}C, ^{1}H] SOFAST-HMQC NMR technique, which subsequently helped us in the design of the next series of small molecule probes.

P-84

PRE-MESSENGER RNA TRANS-SPLICING AS A THERAPEUTIC APPROACH IN DYSFERLIN-DEFICIENT MUSCULAR DYSTROPHY

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The modification of the pre-mRNA *cis*-splicing process by engineered spliceosome-mediated pre-mRNA *trans*-splicing (SmaRT) is an attractive strategy for the *in situ* correction of genes whose careful transcription regulation and full-length expression is determinative for protein function, as it is the case for the dysferlin (*DYSF*, *Dysf)* gene. *DYSF* loss-of-function mutation results in muscular dystrophies mainly manifesting as limb girdle muscular dystrophy 2B (LGMD2B) and Miyoshi muscular dystrophy 1 (MMD1). We established a 3' replacement strategy for mutated dysferlin (*DYSF*, *Dysf)* pre-mRNAs induced by SmaRT by a pre-mRNA *trans*-splicing molecule (PTM). In contrast to previously established SmaRT strategies, we particularly focussed on the identification of a suitable pre-mRNA target intron other than the optimization of the PTM design. By targeting *DYSF* pre-mRNA introns defined by differentially strong 3' splice sites (3'SS), we found that target introns encoding weak 3'SSs were *trans*-spliced successfully *in vitro* in human LGMD2B myoblasts as well as *in vivo* in muscle of *Dysf*(-/-) mice as we demonstrate on RNA and protein level. We identified concordant qualities among successfully targeted *Dysf* introns and previously targeted endogenous introns in other SmaRT strategies that might facilitate a systematic choice of future SmaRT target introns.

P-85

NOVEL OXIME-ETHER LIPIDS FOR siRNA DELIVERY IN BREAST CANCER CELLS

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We have recently reported oxime-ether lipids as promising nucleic acid delivery agents. Although the positive charge in these lipids plays a crucial role, we undertook the present study to evaluate the overall structure of these lipids as it contributes towards siRNA interactions. Oxime-ether lipids containing modifications in the hydrophobic domain chain length, degree of unsaturation and/or bearing polar domain hydroxyl groups were synthesized. The lipids, all containing a quaternary ammonium head group, were fitted with symmetric (lipid 1, C_{14}/C_{14}), non-symmetric (lipid 2, $C_{12}/_{14}$) or long chain unsaturated (lipid 3, $C_{18:1}/C_{18:1}$) hydrophobic domains. In addition, head group-modified lipids containing hydroxyl groups were synthesized with symmetric (lipid 4, C_{14}/C_{14}) or long chain unsaturated (lipid 5, $C_{18:1}/C_{18:1}$) hydrophobic domains. The oxime-ether lipids were examined for their ability to complex with Dicer Substrate RNA (DS RNA). In addition, the resulting complexes were examined for their delivery potential using the human breast cancer cell line (MDA-MB-231). Dynamic light scattering (DLS) experiments showed the average size distribution of the oxime-ether lipid/DS RNA lipoplexes ranged from 80-110 nm in diameter. Using a fluorescence polarization technique, binding experiments revealed that lipids 1, 2, 4 & 5 bind DS RNA with similar affinity. In contrast, binding of lipid 3 was drastically reduced. Using alexa488-labeled duplexes, the transfection efficiency of various lipids was determined in MDA-MB-231 cells. The relative transfection efficiencies for lipoplexes were dependent on the initial lipid/RNA ratios used. However, lipid 5 exhibited superior transfection efficiency at higher concentrations of the lipid. We also noted that lipid 2 (non-symmetric) consistently showed reduced transfection efficiency at all concentrations tested. Experiments with an endosomal marker indicated that the lipid-siRNA complexes enter the cells via the endocytic pathway. Using DS RNA designed against green fluorescent protein (GFP) together with all the lipids, gene silencing activity of GFP was evaluated in MDA-MB-231/GFP cells. Our data show that the introduction of hydroxyl groups to the polar domain of the oxime-ether lipids and incorporation of unsaturation into the hydrophobic domain favor higher transfection and gene silencing in a cell culture system.

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INVESTING CRISPR-CAS9 CONFORMATIONS USING SITE-DIRECTED SPIN LABELING

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In the type II CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) – Cas (CRISPR-associated) system, RNAs derived from the CRISPR locus associate with the Cas9 protein to form a RNA-guided nuclease that cleaves double-stranded DNAs in a sequence-specific manner. The CRISPR-Cas9 system has been adopted for genome engineering in a wide range of organisms, however, mechanisms of its function at the molecular level is unclear. Recent structural and biochemical studies, including a number of crystal structures of apo- and nucleic-acid-bound Cas9, suggest that conformational alterations play essential roles in target selection and cleavage. We are using the technique of site-directed spin labeling (SDSL) to gain understanding of CRISPR-Cas9 complex conformations at various stages of function. SDSL monitors site-specifically attached stable radicals (e.g., nitroxide spin labels) using electron paramagnetic resonance (EPR) spectroscopy, and provides structural (e.g., distance constraints) and dynamic (e.g., motions at the labeling site) information on the parent molecule. Using a nucleotide-independent labeling scheme, we have attached nitroxides at various locations of a target DNA. The labels were found to minimally impact assembly and cleavage activities of the Cas9 complex, and differences in the EPR data measured indicate variations of local environment at different regions of the complex. The work sets the foundation for obtaining information on Cas9 conformations using SDSL, which, in conjunction with other methods, will help dissecting the mechanism of CRISPR-Cas9 function.

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A NEW PUMILIO REPEAT PROTEIN FAMILY FOR PRE-rRNA PROCESSING AND mRNA LOCALIZATION

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Pumilio/*fem-3* mRNA binding factor (PUF) proteins bind sequence specifically to mRNA targets using a single-stranded RNA-binding domain comprising eight PUM repeats. PUM repeats have now been identified in proteins that function in pre-rRNA processing, including human Puf-A and yeast Puf6. This is a role not previously ascribed to PUF proteins. Here we present crystal structures of human Puf-A that reveal a new class of nucleic acid-binding proteins with eleven PUM repeats arranged in an "L"-like shape. In contrast to classical PUF proteins, Puf-A forms sequence-independent interactions with DNA or RNA, mediated by conserved basic residues. We demonstrate that equivalent basic residues in yeast Puf6 are important for RNA-binding, pre-rRNA processing and *ASH1* mRNA localization. Thus, PUM repeats can be assembled into alternative folds that bind to structured nucleic acids in addition to forming canonical eight-repeat crescent-shaped RNA-binding domains found in classical PUF proteins.

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CHARACTERIZATION OF RETROVIRAL RNA STABILITY ELEMENTS

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Rous Sarcoma Virus (RSV) is a simple retrovirus that relies on host cell machinery for replication. Three viral RNAs are generated from the genome, one of which is the full-length, unspliced transcript that serves as the RNA genome for progeny virions as well as the mRNA for Gag and Pol proteins. The transcript contains features uncommon in host cell mRNAs, including a long 3‘ untranslated region (UTR), making it a putative target of the nonsense-mediated mRNA decay (NMD) pathway. However, this viral mRNA is protected against NMD via a small RNA element known as the RSV RNA stability element (RSE). Deletion of the RSV RSE destabilizes the full-length transcript, which gets degraded in a translation and Upf1-dependent manner. Insertion of the RSV RSE after a premature termination codon (PTC) in gag is also capable of rescuing decay. This strongly suggests that the RSE acts as an insulator against NMD.

We conducted a random mutagenesis screen to determine the consensus sequences crucial for this insulator effect. Polypyrimidine tract-binding protein (PTBP1) was identified as a potential candidate that may interact with the RSV RSE to confer RNA stability. This protein was also independently identified in an RNA pull-down and mass spectrometry screen by our collaborators. Mutation of PTBP1 binding sites in the RSV RSE results in destabilization of the full-length transcript, and this destabilization can be rescued by re-introducing exogenous PTBP1 binding sites.

As the NMD-prone features of RSV RNA are common to other retroviruses, we hypothesize that there may be similar RSE-like RNA elements present in other retroviruses as well. Preliminary results suggest that RSE-like elements exist in both HIV and M-MLV that are capable of stabilizing full-length transcripts in a chimeric RSV construct, and we are currently studying these RSE-like elements in their natural context.

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HIGH THROUGHPUT IDENTIFICATION OF FMRP/FXRP-DEPENDENT RIBOSOME STALLING EVENTS

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Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and the leading known genetic cause of autism in humans, affecting approximately 1/4,000 males and 1/8,000 females. In addition to global cognitive impairment, patients with FXS exhibit a wide range of neurological abnormalities, including seizures, hyperactivity, anxiety, deficits in attention and short-term memory, and hypersensitivity to sensory stimuli. This X chromosome-linked disorder is caused by loss of function of the FMR1 gene product, Fragile X mental retardation protein (FMRP), a polyribosome-associated neuronal RNA-binding protein. FMRP represses the translation of its target mRNA transcripts and has been shown to associate with stalled ribosomes on a specific set of target mRNAs in mouse brain. This ribosome stalling has been detected by the observation of FMRP-dependent puromycin-resistant ribosomes on FMRP target mRNAs, measured by qRT-PCR of mRNA levels in sucrose gradient fractions in the presence or absence of functional FMRP. However, analysis of these FMRP-dependent ribosome stalling events has so far been limited to a few hand-selected targets due to the low-throughput nature of qRT-PCR.

Here, we use RNAseq to identify thousands of ribosome stalling events in mammalian cells dependent on the presence of FMRP and its paralogs, FXR1P and FXR2P. We show that the population of transcripts found to undergo ribosome stalling is highly correlated to that of FMRP-bound transcripts obtained via high-throughput sequencing of RNA from crosslinking and immunoprecipitation (HITS-CLIP). We do observe, however, transcripts which are HITS-CLIP targets of FMRP, but do not exhibit FXRP-dependent ribosome stalling and those which are not HITS-CLIP targets, but do exhibit FXRP-dependent ribosome stalling. These results suggest that RNA deep sequencing significantly improves our capacity to detect FMRP/FXRP-dependent translational control in an unbiased manner, including transcripts of low abundance and of unexpected biological significance. The high-throughput identification of FMRP-dependent ribosome stalling events adds a statistically relevant functional dimension to the existing HITS-CLIP binding data, strengthening the validity of the CLIP target designation while allowing for new biological discovery.

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NEISSERIA MENINGITIDIS CAS9 COULD FUNCTION AS A CRISPR-RNA-GUIDED, tracr-RNA-INDEPENDENT ssDNA CLEAVING ENDONUCLEASE

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CRISPR-Cas is a recently discovered set of pathways that inactivate foreign DNA/RNA in bacteria or archaea by sequence-specifically cleaving the intruding DNA/RNA. The CRISPR-Cas systems thus constitute an adaptive immune system in bacteria and archaea. Currently, Type II-A CRISPR systems (Cas9 protein along with a guide RNA) from *Streptococcus pyogenes* (Sp) and *Streptococcus thermophilus* (Sth) are being widely used in genome engineering for a wide range of targets. CRISPR systems require short sequence motifs called Protospacer Adjacent Motifs (PAM) for directing the DNA targeting event, and different Cas9 orthologs vary in their PAM sequence requirements. There is still scope for improvement in the efficiency of targeted DNA cleavage by CRISPR-Cas9 systems by characterizing different Cas9 orthologs. This is due to the potential to find CRISPR systems with improved targeting efficiency and also to expand the genomic contexts that can be accommodated in genome engineering due to the different PAM requirements for different Cas9 orthologs. Previous work from our group has shown that the Type II-C Cas9 from *Neisseria meningitidis* (NmCas9) has a unique crRNA maturation pathway where the 5' end of the crRNA is transcribed from promoters implanted within the repeat region, and that this crRNA is efficient in interference without the processing that occurs normally in the presence of Cas9 and a trans-acting RNA (tracrRNA) (1). In an accompanying work, we showed that NmCas9 could be efficiently used for engineering human pluripotent stem cells, adding to the CRISPR-Cas genome-engineering repertoire another enzyme with different DNA targeting requirements (2). In the present work, we further characterize the features of NmCas9 by conducting *in vivo* and *in vitro* studies in parallel. Our results show that like other Cas9 enzymes that have been characterized previously, NmCas9 uses an RNA-dependent DNA targeting mechanism, through two independent endonuclease domains (RuvC and HNH) that cleave the non-complementary and complementary strands of the double stranded target DNA, respectively. HNH nuclease domain is relaxed in terms of its divalent metal requirements for DNA cleavage, and in the ability to accept ssDNA substrate. The highlight of our work that differentiates NmCas9 from previously characterized Cas9 orthologs is that NmCas9 can efficiently cleave ssDNA *in vitro* in a crRNA-mediated, tracrRNA-independent manner. Studies on the PAM sequence requirement show that NmCas9 has a strong preference for G in the first position of its PAM sequence (GATT), that the PAM sequence is important for both complementary and non-complementary DNA strands, and that its tolerance for deviations at non-G PAM positions are governed by complex rules both *in vitro* and in bacteria. In summary, our work defines unique features of NmCas9 and may suggest functional advantages of Nm cells to be able to interfere with single stranded intruder DNA.

(1). Zhang *et al*., Mol. Cell. 2013, 50 (4), 488-503.
(2). Hou *et al*., Proc. Nat. Acad. Sc. 2013, 110 (39), 15644-15649.

P-91

ISO-SEQ: IDENTIFICATION OF FULL-LENGTH, ALTERNATIVELY-SPLICED TRANSCRIPTS USING PACBIO SEQUENCING

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Transcriptome analysis has illustrated that the splicing of individual mRNAs can be highly divergent, often resulting in different protein isoforms. Further, mRNA 5' and 3' untranslated regions (UTRs), which can be variable, have been demonstrated to play an important role in post-translational gene regulation. Advances in gene expression characterization and detailed profiling of transcriptomes have been driven over the past few years by high-throughput next generation sequencing technologies. However, the underlying sequence reads produced by these platforms are at most only a few hundred base pairs in length. These technologies therefore rely on sequence analysis software to predict the full-length transcript structure. Accurate gene structure prediction, however, becomes challenging or impossible for very long transcripts with multiple alternatively-spliced exons. The Pacific Biosciences SMRT DNA sequencing platform (PacBio), with its long read length and single-molecule sequencing characteristics, is an ideal system for the analysis of these transcript variations, since it is capable of producing full-length reads spanning the entire transcripts without the need for assembly. We will discuss how we have utilized this platform to examine transcript variation across the whole transcriptome of human cancer cell lines, and by targeting a specific gene via PCR. We have also compared our PacBio sequencing results with those produced using the Illumina sequencing platform. We have discovered a range of alternative splicing and UTR variation well beyond what is reported in annotation databases or the wider literature. Techniques and tools we have developed to maximize the output of a sequencing run, and thus reduce the overall cost of a given experiment, also will be discussed.

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DECIPHERING REGULATED RNA DECAY BY THE KINASE-LINKED SENSOR RNase L

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RNA decay is a hallmark mechanism for regulating gene expression in human cells. Double-stranded RNA (dsRNA) is a potent pathogen and damage-associated stress signal that elicits RNA decay in all mammals. This decay is activated by a family of dsRNA-sensing oligoadenylate synthetases (OAS1/2/3), which produce signaling 2'-5'-linked oligoadenylates (2-5A). Accumulation of 2-5A triggers RNA cleavage by activating the ubiquitous kinase-linked endoribonuclease, RNase L, which serves as the 2-5A receptor. RNase L has key roles in antiviral and antibacterial defense, apoptosis, cell cycle, growth, insulin sensitivity and adipocyte differentiation. Despite these important roles, the basis for selection of RNA targets by RNase L remains unclear. To define the mechanism of RNA cleavage, we solved the crystal structure of human RNase L with 2-5A and an RNA substrate. These findings and complementary biochemical studies suggest a unified mechanism for regulated RNA decay by RNase L and by its sister protein, Ire1, which drives the unfolded protein response (UPR) and regulated Ire1-dependent decay (RIDD).

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RNA POLYMERASE III TERMINATION MUTANTS IN RPC1 AND OTHER SUBUNITS CLUSTER IN THE COMPOSITE CATALYTIC CENTER

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How the active center of a eukaryotic RNA polymerase switches from elongation to transcript release mode at termination is unknown. While for pols I, II & III the cis-sequence elements that trigger the first phase of termination for are quite different, to what degree they may share mechanisms in later phase of transcript and template release, is unknown. Similar to other multisubunit RNA polymerases, the two largest subunits of pol III, Rpc1 (C1) and Rpc2 (C2, a.k.a. Ret1) together form the active center. However, various RNA polymerase-associated subunits are known to extend into and modify the active center of the corresponding enzyme, contributing to initiation, elongation and/or termination. Due to the relative simplicity of its termination signal, oligo(dT), and mechanism, pol III is being characterized for the final stage of termination, disengagement of the active center and release of the 3' oligo(U)-containing nascent transcript. Our laboratory has been mapping mutants that impair pol III termination using a panel of functional suppressor-tRNA gene reporters in S. pombe (that suppress a premature nonsense codon in ade6-704 with red→white colony output) that monitor two aspects of termination, read-through beyond the oligo(dT) signal and 3' oligo(U) heterogeneity. We previously reported mutants that map to highly specific motifs of the C11 and C37 subunits, both of which physically extend into the pol III active center and affect either one or both aspects of termination. Here we report isolation and characterization of C1 mutants derived from a random mutagenisis screen in strains bearing oligo(dT) terminators of varying strength that report if pol III fails to terminate and instead reads through. A minor number of the single-mutation mutants map to the C11-interacting domain of C1 near the funnel and exhibit mild phenotypes. The majority have strong phenotypes and cluster map to two conserved motifs in the active center. We also characterized the mutants for production of nascent intron-containing pre-tRNAs as well as terminator read-through transcripts *in vivo*. With regard to physiological significance, the data indicate that despite a model of facilitated recycling that links termination to reinitiation and might predict that termination deficiency would decrease transcription output, the strongest mutants show no decrease in apparent transcript initiation relative to controls.

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OPTIMIZATION OF *IN VIVO* GENOME-WIDE STRUCTURE PROBING OF RNA FOR RICE PLANTS

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As the structure of RNA often is essential to RNA function[1,2], it is imperative to understand the structure of RNA. While it is possible to do this on a small scale for one or a few RNAs, in order to identify trends in the RNA structure-function relationship, genome-wide observations must be made. Additionally, it is important to make these observations through *in vivo* methods, as *in vivo* and *in vitro* structures can differ radically[3,4]. We are particularly interested in understanding RNA structure-function relationships in the important crop species *Oryza sativa* (rice). To accomplish this task, the chemical dimethyl sulfate (DMS) is applied to 14-day-old rice plants. DMS is able to penetrate cells and methylate the Watson-Crick face of all solvent accessible adenosines and cytosines, and thus globally tag the solvent accessible regions *in vivo*. In our Structure-Seq method[3], reverse transcription (RT) is used to read out this modification, as RT will stop one nucleotide prior to the methylated base. RT is then paired to a ligation mediated polymerase chain reaction (LM-PCR) to allow high-throughput sequencing and identification of globally important structure-function relationships. In an effort to further optimize Structure-Seq, various steps throughout the process, including RT and ligation, were adjusted to improve yield of useful reads and reduce ligation bias. Optimization of these steps will ensure the highest quality data to reveal novel trends in rice RNA structuromes.

1 Sharp, P. A. The centrality of RNA. *Cell* 136, 577-580, doi:DOI 10.1016/j.cell.2009.02.007 (2009).
2 Cruz, J. A. & Westhof, E. The dynamic landscapes of RNA architecture. *Cell* 136, 604-609, doi:DOI 10.1016/j.cell.2009.02.003 (2009).
3 Ding, Y., Tang, Y., Kwok, C. K., Zhang, Y., Bevilacqua, P. C. & Assmann, S. M. *In vivo* genome-wide profiling of RNA secondary structure reveals novel regulatory features. *Nature* 505, 696-700, doi:10.1038/nature12756 (2014).
4 Kwok, C. K., Ding, Y., Tang, Y., Assmann, S. M. & Bevilacqua, P. C. Determination of *in vivo* RNA structure in low-abundance transcripts. *Nature Communications* 4, 2971, doi:10.1038/ncomms3971 (2013).

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REGULATING CELL FATE SPECIFICATION BY mRNA ZIPCODE MASKING: CONSEQUENCES FOR CANCER AND METASTASIS PROGRESSION

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The purpose of this study is to investigate the extent to which spatially regulating β-actin gene expression controls epithelial-mesenchymal fate specification in healthy and cancer tissue culture models. We utilize mRNA zipcode antisense oligonucleotide masking of the β-actin transcript and translation site imaging to assess the consequences of perturbing the location of monomer synthesis during cancer and healthy epithelial tissue establishment and maintenance. Additionally, we developed a novel method to quantify adherens junction assembly based on fluorescence covariance between E-cadherin and F-actin during Ca^{2+} switch experiments to assess the consequences of β-actin monomer synthesis mislocalization in epithelial tissue culture model systems. Using these methods we demonstrate that perturbing the location of β-actin monomer synthesis by mRNA zipcode antisense oligonucleotide masking in healthy 2D and 3D epithelial tissue culture models perturbs adherens junction assembly and causes epithelial-mesenchymal transition. Moreover, perturbing E-cadherin expression or function causes β-actin monomer synthesis mislocalization, perturbs adherens junction assembly, and causes epithelial-mesenchymal transition. By contrast, β-actin mRNA zipcode antisense nucleotide masking in 3D colon cancer tissue culture models causes no additional structural defects since adherens junctions are already disorganized. Importantly, western blot analysis of whole tissue lysates from these colon cancer models reveals altered expression of key components of the pathway used to regulate the spatial location of β-actin monomer synthesis such as E-cadherin or Zipcode Binding Protein-1. Together, these data support a model where E-cadherin regulates the location of β-actin monomer synthesis by modulating Zipcode Binding Protein-1/mRNA zipcode interactions to control adherens junction homeostasis and consequently epithelial-mesenchymal cell fate specification. Using β-actin antisense zipcode oligonucleotide masking during Ca^{2+} switch experiments we demonstrate 3D colon cancer tissue culture models have already lost function of the β-actin spatial translation pathway and therefore are not further perturbed during this assay. Consequently, we hypothesize that spatially regulating β-actin monomer synthesis is a key aspect of controlling epithelial-mesenchymal cell fates specification and loss of this pathway is expected to predispose epithelial tissues to cancer and metastasis progression. This is an attractive hypothesis explaining the molecular and cellular mechanism of how E-cadherin and Zipcode Binding Protein-1 function as potent metastasis inhibitors and highlights the importance of spatially regulating β-actin gene expression as an approach to establish and maintain healthy epithelial tissues.

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DEVELOPMENT OF A Cu(II)-BASED SHIFT REAGENT TO STUDY RNA-PROTEIN INTERACTIONS USING IN-CELL NMR TECHNIQUES

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Development of a Cu(II)-based NMR shift reagent will be described. The shift-reagent allows to study RNA-protein interactions in live cells using in-cell NMR techniques. During the past decade, in-cell NMR spectroscopy has evolved as a powerful technique to study three-dimensional structure of biomolecules localized to their native environments. Two-dimensional correlation experiments, such as heteronuclear single quantum coherence (HSQC) and heteronuclear multiple quantum correlation (HMQC), are utilized for the detection of pairs of coupled nuclei. The vast majority of the reported studies focused on mapping protein structures inside the cell. Meanwhile, there is a growing interest to investigate RNA-protein interactions. The NMR shift reagent consists of a Cu(II)-cyclen complex 'clicked' to the RNA sequence of interest. The paramagnetic metal causes bleaching of the NMR signals corresponding to the protein nuclei within the 10 Å radius. To examine the scope and properties of the NMR shift reagent we carried out an *in vitro* study involving an HIV-1 virion protein, NCp7, that binds to a pentanucleotide, ACGCU. The last uridine residue of the pentanucleotide was labeled with the shift reagent using 'click' chemistry. Meanwhile, NCp7 was expressed in *E. coli* grown in $^{15}NH_4Cl$-rich media, thereby isotopically labeling the protein's backbone. *In vitro* interaction between the shift reagent-labeled ACGCU and isotopically-labeled NCp7 were studied by HSQC.

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PIWI-DEPENDENT CHROMATIN STRUCTURE OF GENOMIC LOCI IN *DROSOPHILA*

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Silencing of transposons in the Drosophila ovary relies on three Piwi family proteins—Piwi, Aubergine (Aub), and Ago3—acting in concert with their small RNA guides, the Piwi-interacting RNAs (piRNAs). Aub and Ago3 are found in the germ cell cytoplasm, where they function in the ping-pong cycle to consume transposon mRNAs. The nuclear Piwi protein is required for transposon silencing in both germ and somatic follicle cells, yet the precise mechanisms by which Piwi acts remain largely unclear. In the present study we investigated changes in global chromatin structure upon tissue-specific Piwi knockdown in *Drosophila* ovaries. Using high-throughput sequencing methods such as ChIP-seq, GRO-seq, smRNA-seq and RNA-seq allowed us to understand a new role of Piwi in transcriptional gene silencing in addition to its known functions.

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ANALYSIS OF miR-21 isomiRs IN LUNG CANCER

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Background: microRNAs (miRNA) are small non-coding RNAs that target mRNA transcripts. Primarily, they bind to the 3'UTR of mRNA sequences and repress protein translation. They are involved in the regulation of over 60% of human genes. miRNAs are deregulated in cancer and expression signatures have been correlated with cancer diagnosis, progression and prognosis. Thus, they are being pursued for clinical application. isomiRs, or miRNA variants that are generated post-transcriptionally through the action of DICER, were originally classified as experimental error in cloning and next generation sequencing studies. However, over the past five years it has become increasingly clear that isomiRs are evolutionarily conserved, prevalent and functional. miR-21 is overexpressed and associated with poor prognosis in multiple malignancies and as such it is currently under development as a therapeutic target for several cancers, including lung cancer. We hypothesized that the isomiR landscape of miR-21 is an important element of how this miRNA functions and will be important to consider in the development of therapeutic strategies.

Study Design and Methods: We examined the miR-21 isomiR landscape in lung cancer using data from The Cancer Genome Atlas (lung adenocarcinoma n=420 and squamous cell carcinoma n=296). Raw sequence files were extracted from TCGA, cleaned and analyzed using STATA 13.

Results and Conclusions: We observed that there are significantly more isomiRs of miR-21 in both lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) compared with non-involved adjacent lung tissue. Specifically, LUAD had total of 244 5p variants and LUSC had 232 5p variants, compared with 74 and 105 in non-involved tissue, respectively. Interestingly, the most prevalent isomiR of miR-21 was not the canonical 'miRBase' isomiR and the most common edit was a 5' deletion with 3' addition. Fifteen miR-21-5p isomiRs were expressed at significantly higher levels in LUAD compared with non-involved tissues, compared with LUSC. These were isomIRs that were both prevalent (defined as detected in more than 50% of patients) and abundant (defined as detected with a FPKM value greater than 50). The average fold change was 5.7 in LUAD (range 2.8-21.1) and 2.7 in LUSC (range 1.7-5.6). One isomiR was 20 fold higher in tumor compared with non-involved tissue. Of the prevalent miR-21-5p isomiRs, 6 in LUAD and 4 in LUSC had a change in the miR-21 seed sequence. An analysis of the targets of these isomiRs using the target prediction algorithms Targetscan and Diana revealed limited overlap in gene targets (0% to 54%) compared with canonical miR-21-5p targets, suggesting these isomiRs have different target repertoires.

Relevance and Importance: Our work highlights an extraordinary complexity of miRNA sequence heterogeneity in lung cancer. Combining the characterization of miR-21 isomiRs and pursuing translational laboratory studies will allow us to determine the functionality of these isomiRs and their relevance for human cancer diagnosis and precision treatment.

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NOVEL FUNCTION FOR A NUCLEOPORIN IN miRNA PATHWAY

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Nucleoporins (Nup) are protein components of the nuclear pore complex (NPC). Nup358 is a nucleoporin present on the cytoplasmic side of the NPC, and it also accumulates in the cytoplasm as distinct puncta in annulate lamellae (AL), which are stacked endoplasmic reticulum with pore-like structures. In addition to Nup358, a subset of other nucleoporins is also present in AL. Though a lot of electron microscopic studies have been done, a specific function for AL or AL-associated nucleoporins is unclear. We observed that Nup358-positive AL structures were often present juxtaposed to two mRNA containing cytoplasmic structures; processing bodies (P bodies) and stress granules (SGs). P bodies are involved in microRNA (miRNA)-mediated suppression /degradation of mRNAs, while SGs contain translationally blocked mRNAs at the pre-initiation complex and are formed only when cells are subjected to mild stress. The physical association of AL with these structures indicates a possible role of Nup358 and / or AL in some aspects of mRNA regulation. miRNAs are small non-coding RNAs of around 21-25 nucleotide length, which in association with Argonaute (Ago) forms miRNA induced silencing complex (miRISC). mRNAs, which possess complete / partial sequence complementarity to the miRNAs, are targeted by miRISC for translational suppression or degradation. Interestingly, depletion of Nup358 led to disruption of distinct P body foci in mammalian cells and a concomitant impairment in miRNA-mediated suppression as monitored by reporter assays. This could be rescued by ectopic expression of GFP-Nup358, but not GFP-control. Nup358 depletion did not cause a gross effect on mRNA or miRNA export. Also, miRNA maturation and Ago loading of miRNAs were unaffected in the absence of Nup358. Further studies suggested that Nup358 is specifically required for the association of target mRNAs with miRISC. Consistent with this, when coupling of mRNA with miRNA was by-passed using Ago2-tethering assay, depletion of Nup358 had no significant effect on the target mRNA suppression. These findings uncover a novel function for Nup358 in miRNA pathway.

P-100

EXPLORING THE ROLE OF tRNA MODIFICATION IN MITOCHONDRIAL TRANSLATION REGULATION

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Mitochondria, often termed the 'power house' of the cell, produce over 90% of the energy used by mammalian cells in the form of ATP. In addition, these organelles play a role in a wide range of cellular functions and signaling events such as, heme and steroid synthesis, the storage of intracellular calcium ions, the regulation of cellular proliferation, and programmed cell death. Mitochondrial deregulation and damage has been linked to human aging and aging-related diseases. Mitochondria contain their own DNA and a translational machinery to produce the proteins needed for the generation of ATP by oxidative phosphorylation. The RNA components necessary for mitochondrial translation are exclusive to mitochondria though proteins are often imported from the cytoplasm. Mitochondrial tRNAs (mtRNAs) are an important component of the mitochondrial translation machinery. Like cytoplasmic tRNAs modified nucleosides are a characteristic structural feature of mtRNAs and are required for the correct folding and functioning of mtRNAs. MtRNAs contain 16 species of modified nucleosides, including three mitochondria-specific modifications: 5-formylcytidine, 5-taurinomethyluridine, and 5-taurinomethyl-2-thiouridine. This is the first example for taurine, a naturally occurring sulphonic acid, to be present as a component of biological macromolecules. Though a myriad of studies have established taurine as an important factor in coronary artery disease, blood pressure, plasma cholesterol and myocardial function in animal models of human disease the mechanism behind these observations have been less clear. We hypothesize that loss or lack of taurine in the cell will change the modification status of mtRNAs. Since these taurine modifications are present at the wobble position of mtRNAs, loss of these modifications will in turn affect their translation capabilities. Lack of correctly folded and fully functional mtRNAs will cause deregulation of mitochondrial translation. Accumulation of misfolded proteins in the mitochondrial matrix will then activate mitochondrial UPR (UPR^{mt}) and other stress response pathways. We are using a mouse model for our studies. These mice lack the enzyme Cysteine dioxygenase (CDO) which coverts cysteine into taurine and hence contain very low levels of hepatic and plasma taurine. We are performing a mitochondria specific ribosome profiling (MSRP) in the liver tissue of these mice to study the effect of taurine on mitochondrial translation.

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INDEPENDENT REGULATION OF TRANSCRIPTION AND ALTERNATIVE PRE-mRNA SPLICING BY A HISTONE METHYLTRANSFERASE

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Recent evidence points to a role of chromatin in regulation of alternative pre-mRNA splicing (AS). In order to identify novel chromatin regulators of AS, we screened an RNAi library of chromatin proteins using a cell-based high-throughput *in vivo* assay. We identified a set of chromatin proteins that regulate AS. Using simultaneous genome-wide expression and AS analysis, we demonstrate distinct and non-overlapping functions of these chromatin modifiers on transcription and AS. Detailed mechanistic characterization of one dual function chromatin modifier, the H3K9 methyltransferase G9a, identified VEGF as a major chromatin-mediated AS target. Silencing of G9A, or its heterodimer partner EHMT1, affects AS by promoting exclusion of VEGF exon 6a but does not alter total VEGF mRNA levels. The epigenetic regulatory mechanism of AS by G9A involves an adaptor system consisting of the chromatin modulator HP1γ which binds methylated H3K9 and recruits splicing regulator SRSF1. The epigenetic regulation of VEGF is physiologically relevant since G9A is transcriptionally induced in response to hypoxia and triggers concomitant changes in AS of VEGF. These results characterize a novel epigenetic regulatory mechanism of AS and they demonstrate separate roles of epigenetic modifiers in transcription and alternative splicing.

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HFQ C-TERMINUS AFFECTS RNA BINDING AND ANNEALING VIA RIM INTERACTIONS

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Hfq is a bacterial RNA chaperone which post-transcriptionally regulates mRNAs involved in many cellular processes including metabolism, stress responses and virulence. Hfq binds to small RNAs (sRNAs) and mRNA leader sequences, increasing the rate of base-pairing and stabilizing an sRNA-mRNA complex. Positively charged, arginine-rich patches on the rim of Hfq are important for annealing activity. Access to these positively charged patches may be controlled in order to decrease the propensity of non-specific binding to negatively charged phosphate backbones of RNAs which are not regulated by Hfq. The disordered C-terminus of Hfq varies greatly in length and sequence, however an enrichment of acidic residues is seen at the C-terminal tips of γ-proteobacteria Hfqs. Small Angle X-ray Scattering and fluorescence anisotropy showed that the positive charge at the rim is necessary for binding of the C-terminus by the rim annealing sites. Molecular beacon annealing assays show that the C-terminal tail inhibits binding and annealing of short RNAs *in vitro*. Co-transcriptional annealing of sRNAs to mRNAs *in vitro* shows that the C-terminal tail assists the annealing of certain sRNA-mRNA pairs. Overall, our results suggest that the negatively charged tip of the C-terminus binds to the positively charged rim patches of Hfq and affects RNA binding and annealing *in vitro*.

P-103

CAJAL BODIES SHAPE GENOME CONFORMATION

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Prevailing thoughts regarding the nuclear domain known as the Cajal body (CB) propose a reactionary, passive region which catalyzes the biogenesis of small nucle(o)lar RNAs (sn(o)RNAs) and recycling of essential spliceosomal components after splicing. This domain, which is primarily found in aneuploid transformed cells, is known to specifically form at sites of small RNA transcription and has been observed to interact with several classes of snRNA genes. We hypothesized that CBs may cluster small RNA genes from several chromosomes simultaneously, as well as histone genes through the physically associated Histone locus body, providing an optimized environment for target RNA production and spliceosome assembly. Here, we examined the non-random positioning of CBs, major nuclear bodies involved in efficient spliceosomal assembly, and their role in human genome organization.
We find that CBs are predominantly located at the periphery of chromosome territories at the interface of multiple chromosomes. Genome-wide chromatin conformation capture analysis (4C-seq) revealed that CB-associated regions are enriched in highly expressed genes, including histone loci, and all categories of small U RNA loci. CB-associated genes form inter-chromosomal gene clusters in the 3D space. Analysis of the CB associated with chromosome 1 showed that the CB is necessary for configuring the entire chromosome into a rosette-like structure which envelopes the CB. RNAi-mediated disassembly of CBs leads to a substantial disruption of the CB-targeting gene clusters and widespread suppression of small U RNA and histone genes. We also observed discrete changes in gene expression and global increase in splicing noise even outside of the CB-proximal genomic regions.
We conclude that CBs are not passive byproducts of specific gene activity, but play an active role in organizing the genome in 3D, likely to accelerate snRNA processing steps but also to influence the expression and splicing of RNA pol II-driven genes in Cajal body proximal regions.

P-104

ESCHERICHIA COLI SIGMA 38 PROMOTERS USE TWO UP ELEMENTS INSTEAD OF A −35

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In *E. coli*, one RNA polymerase (RNAP) is responsible for transcription of all RNA species; however, different regulons are recognized by RNAP containing different sigma factors. RNAP containing sigmaS (sigma38) is responsible for the expression of genes responding to stress conditions such as stationary phase or osmotic shock. In order to begin construction of a model of sigma38 promoters, we aligned proven transcriptional starts recorded in RegulonDB. We then shuffled the sequences to maximize the information upstream of the start and identified a −10 similar to that in sigma70 promoters. Because activators can replace the −35, alignment of the −35 region of sigma70 promoters is difficult, but in the case of sigma38 promoters we could not identify any −35 pattern at all. However, we found two patterns upstream of the −35 region. These patterns are complementary and correspond to the location of UP elements bound by the polymerase αCTD in ribosomal promoters. Essentially all sigma38 promoters can be characterized this way. We propose that sigma38 promoters do not use a −35 but use two UP elements instead. We are planning experiments to test this model.

P-105

IMPROVING PREDICTIONS FOR TERMINAL MISMATCHES IN RNA STRUCTURES AND ENERGETICS

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The power of RNA to regulate gene expression has the potential to cure cancer. The energetics of RNA duplexes and imperfect matches play an important role in the specificity and efficacy of microRNAs that regulate expression of cancer genes and of RNA therapeutics with the potential to cure cancer. Understanding the energetic stability of mismatches in RNA helices is a key step toward understanding off-target effects of small RNA therapeutics. Energetic stabilities form the foundation for RNA structure predictions, which are a useful tool for determining microRNA target site accessibility.

This research focuses on improving the predictions of terminal mismatches that occur in small RNA-mRNA duplexes. The thermodynamic stabilities of consecutive terminal mismatches are more stable than previously predicted. The new predictions improve the correlation between thermodynamic stabilities and effective gene silencing by an siRNA for chemokine receptor 4 (CXCR4) gene. The benchmarks for RNA duplex stabilities in different salt concentrations and molecular crowding conditions improve predictions in experimental conditions. The enthalpic and entropic contributions to consecutive terminal mismatch motifs were studied using substitutions with locked nucleic acid adenosines. Consecutive terminal GU pairs show the most sequence-dependent stabilities. NMR, crystallography (1.3 Å resolution), and molecular modelling studies show cross-strand G stacking that explains the sequence dependence of this motif. Duplexes with four consecutive terminal GU pairs form surprisingly A-form-like helices that could be recognized by RNA binding proteins.

These results contribute to the ongoing improvement of the thermodynamic database of RNA motifs that forms the core of RNA structure prediction tools. Accurate structure predictions will be essential for discovering the complex network of RNA interactions that regulate gene expression in cancer biology. This new terminal GU pair structural motif provides insight into RNA structure-energetics relationships and will facilitate *de novo* predictions of RNA structure from sequence and fundamental physical principles. Consecutive terminal GU pairs may have previously been underpredicted. Thus, these results will aid future searches for functional sites in non-coding RNAs and target sites of small RNAs in gene regulation.

"The Effects of Salt, Polyethylene Glycol, and Locked Nucleic Acids on the Thermodynamic Stabilities of Consecutive Terminal Adenosine Mismatches in RNA Duplexes." X. Gu, M.-T. Nguyen, A. Overacre, S. Seaton, S.J. Schroeder. *J. Phys. Chem. B.* 117, 3531-3540 (2013).
"Consecutive Terminal GU Pairs Stabilize RNA Helices." M.-T. Nguyen, S.J. Schroeder. *Biochemistry*. 49, 10574-10581 (2010).
"3' Terminal Nucleotides Determine Thermodynamic Stabilities at the Ends of RNA Helices," K. Clanton-Arrowood, J. McGurk, S.J. Schroeder. *Biochemistry*, 47, 13418-13427 (2008).
"Structural insights into how consecutive G·U wobble base pairs stabilize the ends of RNA helices" X. Gu, B. Mooers, L. Thomas, J. Malone, S. Harris, S.J. Schroeder. submitted.

P-106

PROLONGED REGULATION OF microRNA BIOGENESIS UNDERLYING NEURONAL GROWTH

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Enduring memory formation requires changes in neural gene expression; but how selected genes are appropriately regulated is incompletely understood. Post-transcriptional regulation is now known to be critical in determining cellular protein levels, highlighting the importance of revealing molecular mechanisms that can orchestrate pro-growth programs of gene expression post-transcriptionally. Brain-derived neurotrophic factor (BDNF) has received widespread attention for its role in modulating protein synthesis during neuronal plasticity and learning, as well as for its remarkable gene target specificity. Our lab previously demonstrated that rapid transcription-independent, BDNF-induced target specificity depends upon dual regulation of miRNA biogenesis: 1) BDNF enhances Dicer to promote miRNA biogenesis and widespread translational repression and 2) BDNF elevates Lin28a protein levels to prevent processing of select miRNAs, primarily the Let-7 family. Let-7 miRNAs inhibit translation of many pro-growth and plasticity-related mRNAs, and loss of let-7 miRNAs following BDNF relieves suppression of these genes.

In ongoing work, we are testing the hypothesis that long-term maintenance of gene target specificity in neuronal gene expression relies upon prolonged regulation of microRNA biogenesis. Preliminary studies show that transient BDNF stimulation of murine hippocampal slices induces persistent regulation of miRNA levels, including the Let-7 family, as well as protein components of miRNA biogenesis pathways. Current studies investigate the transcriptional and post-transcriptional mechanisms responsible for these observations, including the possibility of an auto-regulatory post-transcriptional feedback loop involving the Lin28/Let-7 axis, which may permit prolonged regulation of miRNA biogenesis following BDNF exposure. Future experiments will test the roles of the Lin28/Let-7 axis on BDNF-induced changes in synaptic plasticity, neurophysiology, and *in vivo* cognitive tasks.

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P-107

MECHANISMS AND EVOLUTIONARY CONSERVATION OF RNA RECOGNITION AND CATALYSIS BY THE *ESCHERICHIA COLI* RNA PYROPHOSPHOHYDROLASE RppH

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Bacterial RNA degradation often begins with conversion of the 5′-terminal triphosphate to a monophosphate by the RNA pyrophosphohydrolase RppH, an event that triggers rapid ribonucleolytic attack. Besides its role as the master regulator of 5′-end-dependent mRNA decay, RppH is important for the ability of pathogenic bacteria to invade host cells, yet little is known about how it chooses its targets. We show that *Escherichia coli* RppH (EcRppH) requires at least two unpaired nucleotides at the RNA 5′ end and prefers three or more. It can tolerate any nucleotide at the first three positions but has a modest preference for A at the 5′ terminus and either G or A at the second position. To understand the mechanism of RNA recognition and catalysis, we determined the X-ray structures of EcRppH in apo and RNA-bound forms. These structures show distinct conformations of EcRppH-RNA complexes on the catalytic pathway and suggest a catalytic mechanism that may be shared by other members of the Nudix hydrolase superfamily. EcRppH interacts with RNA by a bipartite mechanism involving specific recognition of the 5′-terminal triphosphate and the second nucleotide, thus enabling discrimination against mononucleotides as substrates. The structures also reveal the molecular basis for the preference of the enzyme for RNA substrates bearing guanine in the second position by identifying a protein cleft in which guanine interacts with EcRppH side chains via cation-π contacts and hydrogen bonds. Structure-guided mutational analysis indicates the contribution of each amino acid of the EcRppH RNA-binding site to the specificity and affinity of mRNA recognition. Together, our structural and biochemical data explain the modest sequence specificity of EcRppH and its stringent requirement for at least two unpaired nucleotides at the 5′ terminus. The relative promiscuity of this enzyme distinguishes it from the highly sequence-selective RppH present in *Bacillus subtilis* (BsRppH). EcRppH orthologs likely to share its relaxed sequence specificity are present in all classes of Proteobacteria except Deltaproteobacteria, and in flowering plants. By contrast, the phylogenetic range of recognizable BsRppH orthologs appears to be restricted to the order Bacillales. These findings help to explain the selective influence of RppH on bacterial mRNA decay and show that RppH-dependent degradation has diversified significantly during the course of evolution, discoveries with implications for bacterial pathogenesis and other cellular processes dependent on RppH activity.

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DEREGULATION OF HOST LONG NON-CODING RNAs (lncRNAs) DURING LATENT KAPOSI'S SARCOMA ASSOCIATED HERPES VIRUS (KSHV) INFECTION

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Pervasive transcription of the human genome and biological significance of long non-coding RNAs (lncRNAs) are now widely accepted to be of disease relevance. LncRNA deregulation has been implicated in many human diseases, especially cancer. Viral infection is another important context where host lncRNA deregulation has been reported. Interestingly, regulation of lncRNAs via the miRNA-mediated repression pathway has been recently demonstrated by several labs. KSHV being an oncovirus that encodes miRNAs provides us with an appropriate system to study lncRNA deregulation from the perspectives of both cancer and viral infection. KSHV is the etiological agent of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD). KS is the primary AIDS-associated malignancy and accounts for over 50% of the cancers in African males. Latent replication is the tumor-associated replication mode of KSHV. During latency, KSHV expresses 4 major latency proteins and 25 miRNAs. Studies to identify the viral miRNA targetome using HITS-CLIP identified that lncRNAs constituted a significant portion of the targetome suggesting a KSHV-miRNA mediated host lncRNA regulation. Significant amounts of KSHV miRNAs and Ago-2 were detectable in PEL cell nuclei explaining partly the nuclear lncRNAs identified as a part of miRNA targetome. We performed a microarray analysis of host lncRNAs in the contexts of wildtype KSHV and miRNA deleted KSHV infections. A significant deregulation of host lncRNAs was observed in both contexts, including known cancer related lncRNAs like UCA1 and ANRIL. Approximately 25% of the lncRNAs downregulated in wtKSHV infection were rescued in ΔmiRNA-KSHV infection and they are potentially direct targets of KSHV miRNAs. Based on these results, we hypothesize that KSHV encoded latency factors deregulate host lncRNAs and a fraction of that is through direct miRNA-lncRNA interactions. Experiments are underway to address the suggested lncRNA-miRNA interactions and their functional consequences. Functional characterization of some of the disease-relevant novel lncRNAs identified in our datasets would add to the as-yet limited pool of characterized lncRNAs. Importantly, lncRNA-miRNA interaction is a recent development in the ncRNA biology field and KSHV serves as a useful genetic tool to address these in detail (as individual miRNA mutants are available). Deciphering lncRNA function may also contribute towards novel therapeutic targets for cancer and KSHV pathogenesis.

P-109

MULTI-FUNCTIONAL NUCLEIC ACID BASED NANOPARTICLES: THEIR DESIGN, PRODUCTION AND ACTIVATION

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Control over the simultaneous delivery of different functionalities and their synchronized intracellular activation can significantly contribute to the fields of RNA and DNA biomedical nanotechnologies. We present several different, yet related methodologies depicting how one can computationally design, and experimentally assemble and deliver functionalized nucleic acid based nanoparticles that have single or multiple functionalities. First, we present a system consisting of single or multiple split functionalities embedded in related pairs of RNA-DNA hybrids which are programmed to recognize each other, re-associate, and form DNA duplexes that release the split RNA fragments, which upon association regain their original functions. In addition, we show how nanoscaffolds (nanorings) can be functionalized with multiple short interfering RNAs for combinatorial RNA interference, and also permit the simultaneous incorporation of assorted RNA aptamers, fluorescent dyes, proteins, as well as auto-recognizing RNA-DNA hybrids used to conditionally activate multiple split functionalities. These constructs were extensively characterized and visualized *in vitro*, in cell culture and *in vivo* by various experimental techniques. The results also revealed that the use of these functionalized RNA nanostructures promote higher detection sensitivity in diseased cells and significant increases in silencing efficiencies of targeted genes compared to the silencing caused by equal amounts of conventional siRNAs. Finally, we report a generalized methodology for the one-pot production of chemically modified functional RNA nanoparticles during *in vitro* transcription with T7 RNA polymerase. The efficiency of incorporation of 2'-fluoro-dNTP in the transcripts by the wild type T7 RNA polymerase dramatically increases in the presence of manganese ions, resulting in high-yield production of chemically modified RNA nanoparticles functionalized with siRNAs that are resistant to nucleases from human blood serum. Moreover, the unpurified transcription mixture can be used for functional *ex vivo* pilot experiments.

P-110

IDENTIFICATION OF DDSR1: A DNA-DAMAGE INDUCIBLE lncRNA INVOLVED IN HOMOLOGOUS RECOMBINATION

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Long non-coding RNAs (lncRNAs) are important players in diverse biological processes. Upon DNA damage, cells elicit a complex signaling cascade referred to as DNA damage response (DDR). Using a microarray screen we identified a novel lncRNA named as DNA damage sensitive RNA 1 (DDSR1), which is induced upon DNA damage by several double strand break (DSB) agents. DDSR1 induction upon DNA damage is dependent on the ATM-NF-κB pathway. P53 expression induces DDSR1 transcription but is not necessary for DNA damage induced DDSR1 transcription. Gene expression analysis upon DDSR1 knockdown shows it negatively regulates the expression of p53 target genes. Loss of DDSR1 reduces DNA repair capacity by homologous recombination (HR) and promotes aberrant accumulation of BRCA1 at double strand breaks sites. DDSR1 interacts with hnRNPUL1, an RNA-binding protein involved in DNA repair by HR. Our results suggest a role for lncRNAs in modulation of DNA damage repair and signaling.

P-111

THE HIV-1 REV RESPONSE ELEMENT EXISTS IN TWO ALTERNATIVE CONFORMATIONS THAT PROMOTE DIFFERENT RATES OF VIRUS REPLICATION

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The binding of HIV-1 Rev protein to the Rev Response Element (RRE), a *cis*-acting RNA element in all intron-retaining HIV mRNAs, is required for the nucleo-cytoplasmic export of these mRNAs. However, the precise secondary structure of the RRE remains controversial. Studies have reported that the RRE has either 4 or 5 stem-loops, which differ only in the rearrangement of functionally undefined regions of the RRE. To understand the role played by these regions, we have examined the relationship between these two structures and Rev-RRE activity.

In vitro transcribed NL4-3 RRE was found to migrate as a "doublet" band on a native polyacrylamide gel. Using in-gel *Selective 2' Hydroxyl Acylation analyzed by Primer Extension* (SHAPE), we found that one of these bands consisted of an RRE with a 5 stem-loop structure, whereas the other was a 4 stem-loop structure. Thus, our data demonstrate, for the first time, that the NL4-3 RRE exists in two alternative structures.

To study the significance of these alternative structures, we made RRE mutants that were confirmed using SHAPE to allow only one or the other of the structures to form. Analysis of the complexes that each RRE formed with purified Rev protein *in vitro* showed differences in the migration rates of these complexes on native gels, suggesting structural differences. The RREs were also tested for their abilities to promote viral replication, by inserting each RRE into the Nef region of an RRE-defective provirus which contained RRE mutations that do not change the Env protein. Growth kinetics and competition assays showed that the virus with the 5 stem-loop RRE had a higher replicative fitness than the virus with the 4 stem-loop RRE. These results suggest that HIV may use two alternative RRE secondary structures to modulate replication, potentially allowing adaptation to environmental demands in time and/or space.

P-112

MUTATIONS IN SF3B1 LEAD TO ABERRANT SPLICING THROUGH CRYPTIC 3' SPLICE SITE SELECTION AND IMPAIR HEMATOPOIETIC CELL DIFFERENTIATION

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Heterozygous mutations in SF3B1, a component of the U2 complex involved in the recognition of 3' splice sites (ss), have been reported with high frequency in myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL) and several solid tumors. To study the impact of SF3B1 mutations on splicing, RNAseq data obtained from breast cancer, melanoma, CLL and MDS samples with mutant ($SF3B1^{MUT}$) or wild-type SF3B1 ($SF3B1^{WT}$) were compared. The majority of aberrant junctions identified in the samples with mutant SF3B1 utilized an alternative 3'ss, suggesting a neomorphic function. Motif analysis of the sequences used by $SF3B1^{MUT}$ revealed the usage of a cryptic AG 3'ss with a shorter and weaker polypyrimidine tract, features that were validated experimentally using minigenes expressed in $SF3B1^{MUT}$ cell lines. Several aberrant junctions were common across all hotspot mutations and diseases; however, a unique aberrant splicing profile was found for each disease suggesting lineage and disease specific effects. In most cases studied, aberrant junction usage led to introduction of a premature termination codon downstream of the cryptic AG leading to nonsense mediated decay (NMD) of aberrant transcripts and downregulation of gene expression. Gene-set enrichment analysis of aberrantly spliced and differentially expressed genes in $SF3B1^{MUT}$ MDS patient samples revealed genes involved in cell differentiation and epigenetic pathways; processes known to be deregulated in MDS. One feature of MDS is a block in cellular differentiation; to study the role of SF3B1 mutations in differentiation processes we introduced $SF3B1^{MUT}$ in TF-1 cells following erythropoietin stimulation. TF-1 $SF3B1^{WT}$ cells were able to differentiate normally after EPO treatment; however, expression of $SF3B1^{K700E}$ (the most common hotspot mutation found in MDS and CLL) resulted in a block in erythoid differentiation. Interestingly, the differentiation block observed in $SF3B1^{K700E}$ was associated with cytokine independent growth further indicating a gain-of-function for SF3B1 mutations. Initial mining of RNAseq data from $SF3B1^{MUT}$ TF-1 cells highlighted several aberrantly spliced and NMD-downregulated genes previously implicated in MDS. Finally, SF3B1 transduced TF-1 cells were implanted into immunocompromised mice and after several passages an enrichment of TF-1 $SF3B1^{K700E}$ cells was observed, suggesting a growth advantage for $SF3B1^{MUT}$ cells over $SF3B1^{WT}$ cells. These data suggest that the K700E SF3B1 mutation induces aberrant splicing and a resultant block in differentiation and competitive advantage as observed in human MDS.

P-113

SHAPE VISUALIZATION OF RNA-PROTEIN INTERACTION SITES IN LIVING CELLS

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Nearly all RNAs, regardless of function, interact with one or more protein partners in order to function properly. Thus, the characterization of ribonucleoprotein (RNP) complexes is an important step in understanding many biological processes, but it is difficult to predict RNA-protein interactions *de novo*. Current useful approaches for uncovering these interactions are often limited to a single RNA or protein of interest. SHAPE-MaP (selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling) enables robust, unbiased, and highly automated measurement of nucleotide flexibility for all RNAs present in an experiment, and includes estimates of the uncertainty in these measurements. SHAPE reagents are capable of modifying RNA in living cells, allowing for the measurement of RNA flexibility in both protein-free (*ex vivo*) and cellular (*in cellulo*) contexts. We present a straightforward analytical framework that takes advantage of the error estimates intrinsic to SHAPE-MaP to detect RNA structural differences that occur in living cells. We apply this framework to three model RNP complexes: the U1 snRNP, signal recognition particle, and 5S RNP. In each case, all identified interaction sites correspond to known protein binding sites or to protein-associated RNA structural rearrangements. This strategy avoids false-positive difference detection that occurs when measurement uncertainties common in structure probing data are ignored. While our approach cannot identify specific protein binding partners, it does illustrate how SHAPE-MaP reveals multiple protein interaction sites under native cellular conditions for many RNAs simultaneously. As RNA studies expand more routinely to -omics scales, we expect that broadly applicable strategies for identifying interactions between the transcriptome and proteome will become increasingly important.

P-114

TRANSPOSABLE ELEMENT DYNAMICS AND PIWI REGULATION IMPACTS lncRNA AND GENE EXPRESSION DIVERSITY IN *DROSOPHILA* OVARIAN CELL CULTURES

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Piwi proteins and Piwi-interacting RNAs (piRNAs) repress transposable elements (TEs) from mobilizing in gonadal cells. To determine the spectrum of piRNA-regulated targets that may extend beyond TEs, we conducted a genome-wide survey for transcripts associated with PIWI and for transcripts affected by PIWI knockdown in Drosophila ovarian somatic sheet (OSS) cells, a follicle cell line expressing the Piwi pathway. Despite the immense sequence diversity among OSS cell piRNAs, our analysis indicates that TE transcripts are the major transcripts associated with and directly regulated by PIWI. However, several coding genes were indirectly regulated by PIWI via an adjacent de novo TE insertion that generated a nascent TE transcript. Interestingly, we noticed that PIWI-regulated genes in OSS cells greatly differed from genes affected in a related follicle cell culture, ovarian somatic cells (OSCs). Therefore, we characterized the distinct genomic TE insertions across four OSS and OSC lines and discovered dynamic TE landscapes in gonadal cultures that were defined by a subset of active TEs. Particular de novo TEs appeared to stimulate the expression of novel candidate long non-coding RNAs (lncRNAs) in a cell lineage-specific manner, and some of these TE-associated lncRNAs were associated with PIWI and overlapped PIWI-regulated genes. Our analyses of OSCs and OSS cells demonstrate that despite having a Piwi pathway to suppress endogenous mobile elements, gonadal cell TE landscapes can still dramatically change and create transcriptome diversity.

P-115

IDENTIFICATION OF BIOLOGICALLY ACTIVE, HIV TAR RNA-BINDING SMALL MOLECULES USING SMALL MOLECULE MICROARRAYS

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Identifying small molecules that selectively bind to structured RNA motifs remains an important challenge in developing potent and specific therapeutics. Most strategies to find RNA-binding molecules have identified highly charged compounds or aminoglycosides that commonly have modest selectivity. Here we demonstrate a strategy to screen a large unbiased library of druglike small molecules in a microarray format against an RNA target. This approach has enabled the identification of a novel chemotype that selectively targets the HIV transactivation response (TAR) RNA hairpin in a manner not dependent on cationic charge. Thienopyridine 4 binds to and stabilizes the TAR hairpin with a Kd of 2.4 μM. Structure-activity relationships demonstrate that this compound achieves activity through hydrophobic and aromatic substituents on a heterocyclic core, rather than cationic groups typically required. Selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) analysis was performed on a 365-nucleotide sequence derived from the 5' untranslated region (UTR) of the HIV-1 genome to determine global structural changes in the presence of the molecule. Importantly, the interaction of compound 4 can be mapped to the TAR hairpin without broadly disrupting any other structured elements of the 5' UTR. Cell-based anti-HIV assays indicated that 4 inhibits HIV-induced cytopathicity in T lymphocytes with an EC50 of 28 μM, while cytotoxicity was not observed at concentrations approaching 1 mM.

P-116

HIDDEN RNA CODES REVEALED FROM THE PLANT *IN VIVO* RNA STRUCTUROME

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RNA can fold into secondary and tertiary structures which are important for gene regulation. Recently, we have developed a method to perform genome-wide RNA structure profiling *in vivo* employing high throughput sequencing techniques in Arabidopsis [1]. This work makes it possible to probe thousands of RNA structures at one time in living cells. Hidden RNA codes have been revealed by bioinformatics and biostatistics analysis from our *in vivo* RNA structuromes including RNA secondary structures related to alternative polyadenylation and splicing, as well as a correlation between mRNA structure and the structure of the encoded protein. These results indicate the vital roles of RNA structures in regulation of gene expression. A new computational platform, StructureFold, has been developed to facilitate the bioinformatic analysis of high throughput RNA structure profiling data. As a component of the Galaxy platform (http://www.galaxyproject.org), StructureFold integrates reads mapping, RT stop count calculation, reactivity derivation, and structure prediction in a user-friendly web-based interface. StructureFold is an efficient tool for the analysis of high-throughput RNA structural probing data which otherwise requires great bioinformatic efforts. StructureFold can be extended in the future to become a universal bioinformatics tool for studies of RNA structure based on high-throughput sequencing methodologies.

1. Ding Y, Tang Y, Kwok CK, Zhang Y, Bevilacqua PC, Assmann SM: *In vivo* genome-wide profiling of RNA secondary structure reveals novel regulatory features. *Nature* 2014, 505(7485):696-700.

P-117

IDENTIFICATION OF PROTEINS INVOLVED IN miR-338 TRAFFICKING IN DISTAL AXONS OF SYMPATHETIC NEURONS

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mRNA localization and its on-site translation within neuronal subcompartments allow for spatially restricted gene expression and are vital for polarized cells, like neurons. A heterogenous population of mRNAs are localized within the distal axons of primary sympathetic neurons, including nuclear-encoded mitochondrial mRNAs, such as cytochrome c-oxidase (COXIV) and ATP-synthase (ATP5G1). Local synthesis of these mRNAs regulate oxidative phosphorylation and ATP production. In addition to mRNAs, axons contain a diverse population of micro RNAs (miR) that serve to regulate the local synthesis of their target messages. Earlier studies have shown that COXIV and ATP5G1 contain a putative 3'UTR hairpin-loop binding site for miR-338. Axonal transfection of miR-338 decreases the endogenous levels of COXIV and ATP5G1 mRNAs and proteins, and affect axonal bioenergetics and outgrowth. These studies illustrate that miR-338 serve to coordinate the expression of nuclear-endcoded mitchondrial mRNAs in the axon and regulate axonal energy metabolism. However, very little is known regarding the trafficking and maturation of miRs to distal axons.

To identify proteins potentially involved in the trafficking and processing of miR-338, biotinylated precursor and mature miR-338 were used as baits for RNA-protein binding studies using cytosolic proteins isolated from rat whole brain lysates. Gel-retardation assays with precursor and mature miR-338 revealed the presence of specific RNA-protein complexes. Next, the miR-338-bound proteins were purified using streptavidin magnetic bead columns and the identities of the proteins were determined by multidimensional protein identification technology (MuDPIT) using mass spectrometry. Proteomic analysis tools were used to parse out the functional networks of the candidate proteins.

The results showed an enrichment of proteins involved in RNA trafficking and metabolism, organelle and vesicle transport, as well as G-protein coupled and MAPK-dependent signaling complexes. Surprisingly, we also observed an enrichment of mitochondria-associated complexes and the absence of RISC-complex associated proteins.

Future studies will focus on validating the roles of candidate proteins in miR-338 trafficking to the axon using compartmentalized primary neuronal cultures. *In vivo* binding of candidate proteins with precursor-miR-338 will be tested by RNA-immunoprecipitation assays using axonal protein extracts. siRNA-mediated knockdown of the putative targets of miR-338 will be employed to further investigate the role of the candidate proteins in the trafficking and processing of precursor-miR-338 to the axons.

P-118

SPATIO-TEMPORAL REGULATION OF CIRCULAR RNA EXPRESSION DURING EMBRYONIC BRAIN DEVELOPMENT

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Recently, the phenomenon of circular RNA (circRNA) has gone from being perceived as a rare curiosity to having a central regulatory role in RNA metabolism. By adding a new layer of complexity to RNA biology, circRNA may be an integral regulatory entity to develop and maintain multiple distinct mammalian cell-types and organs from the same genetic information. Thousands of circular RNAs (circRNAs) have been discovered in various tissues and cell types from human, mouse and fruit fly. However, expression of circRNAs across mammalian brain development has never been examined. The porcine brain is comparable to the human brain based on anatomy, histology, growth and development, and its size enables further and earlier dissection as compared to rodents. Furthermore, a high quality draft pig genome has recently become available. Here we profile the expression of circRNA in 5 different brain tissues at up to 6 different time points during fetal development, using Illumina deep sequencing of porcine brain samples.

An unbiased analysis reveals variable amounts of circular RNAs, with a distinct spatio-temporal expression profile. The amount and complexity of the circRNA expression was most pronounced in cortex day 60 from gestation. Here we find 4,634 unique circRNAs expressed from 2,195 genes out of a total of 13,854 expressed genes. A large proportion (approx. 20%) of the porcine splice sites involved in circRNA production are conserved in the human and mouse genome. Furthermore, we observed that "hot-spot" genes produce multiple circRNA isoforms, which are often differentially expressed across porcine development. A global comparison of porcine circRNAs revealed that introns flanking circularized exons are longer than average and often have proximal complementary SINE sequence elements, which potentially can facilitate base pairing between the flanking introns. These data show that circRNAs are highly abundant in porcine fetal brain and are likely to serve important functions during mammalian brain development.

P-119

THE ZINC FINGER PROTEIN, Zfp217, REGULATES GLOBAL m6A RNA MODIFICATION TO MAINTAIN AND INSURE A PLURIPOTENT STATE

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Many epigenomic and epitranscriptomal networks must be integrated to maintain self-renewal and pluripotency in embryonic stem cells (ESCs) and to enable induced pluripotent stem cell (iPSC) reprogramming. However, it remains unclear whether components of these networks are functionally shared or interact, and what factors mediate such interactions. Here, we identify zing finger protein 217 (Zfp217) as a master transcription factor in maintenance of the undifferentiated state, by directly activating the transcription of key determinant genes of stem cell identity and by playing an essential role in regulating their m6A RNA methylation levels. Composition of the zfp217 -containing ES cell multi-subunit complex identifies mainly hnRNA binding subunits as well as several chromatin regulatory subunits (i.e. Lsd1, Rcor1, 2, Hdac1 and 2). Hence, depletion of Zfp217 resulted in ES cells with severely compromised self-renewal properties that subsequently differentiate in to additional lineages. Genome-wide analysis involving ChIP-Seq and RNA-seq reveals an overlapping gene regulatory function between Pou5F1, LSD1 and Zfp217 in the ESC genome. Furthermore, Zfp217 strongly interacts with the recently identified m6A methyltransferase Mettl3, and depletion of Zfp217 dramatically increases global m6A RNA methylation levels, to demonstrate that Zfp217 is a primary mediator of Mettl3 function, essential to restrain the methyl transferase activity. Moreover, native RIP-seq and iCLIP experiments indicate that both Zfp217 and Mettl3 share a significant amount of bound transcripts, including key pluripotency genes. Collectively, our findings provide for the first time evidence for a critical and novel role of a transcription factor Zfp217, not just in orchestrating transcription of a pluripotency network of genes, but also for the control of the mRNA epigenetic modification m6A, essential for the execution of gene expression programs during early embryonic development.

P-120

SINGLE MOLECULES UNDER THE MICROSCOPE: FROM RNA SILENCING TO SPLICING TO DNA-DIRECTED ENZYME CASCADES

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Nature and Nanotechnology likewise employ nanoscale machines that self-assemble into structures of complex architecture and functionality. Fluorescence microscopy offers a non-invasive tool to probe and ultimately dissect and control these nanoassemblies in real-time. In particular, my group utilizes single molecule fluorescence resonance energy transfer (smFRET) to measure distances at the 2-8 nm scale, and complementary super-resolution localization techniques based on Gaussian fitting of imaged point spread functions (PSFs) to measure distances in the 10 nm and longer range. I will describe a method for the intracellular single molecule, high-resolution localization and counting (iSHiRLoC) of microRNAs (miRNAs), a large group of gene silencers with profound roles in our body, from stem cell development to cancer. Microinjected, singly-fluorophore labeled, functional miRNAs were tracked at super-resolution within individual diffusing particles. Observed mobility and mRNA dependent assembly changes suggest the existence of two kinetically distinct assembly processes. We are currently feeding these data into a single molecule systems biology pipeline to bring into focus the unifying molecular mechanism of such a ubiquitous gene regulatory pathway. In addition, I will describe how we use smFRET to show that single spliceosomes – responsible for the accurate removal of all intervening sequences (introns) in pre-messenger RNAs – are working as biased Brownian ratchet machines. On the opposite end of the application spectrum, we are utilizing smFRET and super-resolution fluorescence microscopy to monitor enhanced enzyme cascades engineered to self-assemble on DNA origami.

P-121

SPLICING FUNCTION OF MITOTIC REGULATORS LINKS R-LOOP MEDIATED DNA DAMAGE TO TUMOR CELL KILLING

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Splicing inhibitors has been shown to possess strong antitumor activity and the first-in-class small molecule E7107 is under Phase I clinical trial in patients with advanced solid tumors. However, the mechanism behind this specific tumor-killing effect has not been elucidated. Through high-throughput screening, we identified a novel gene, BuGZ, could specifically inhibit the proliferation of tumor cells. We showed that BuGZ interacted with the splicing machinery and are required for pre-mRNA splicing. Similar to inhibition of RNA splicing by pladienolide B, depletion of BuGZ led to increased formation of RNA-DNA hybrids (R-loops). On one hand, R-loop formation led to DNA damage and p53 activation in interphase cells; while on the other hand, during S phase DNA replication, R-loops served as primers for DNA synthesis and caused abnormalities in ploidy. These two aspects of defects combined with lack of DNA damage checkpoint in majority of tumor cells, finally resulted in the mitotic catastrophe and diminish of tumors. Our study demonstrates the possible mechanism behind the antitumor activity of splicing inhibitors and splicing regulators. Hopefully, this would pave the way for the further development of this class of antitumor drugs.

P-122

miRNAS REGULATE SPLICING OF HPV16 RNAS BY INTERACTING WITH RNA *CIS*-ELEMENTS

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miRNAs are non-coding, post-transcriptional regulators that bind to complementary sequences on target mRNAs for translational repression and gene silencing. Recent studies indicate that a group of mature miRNAs exist in the nucleus, implying possible roles of certain nuclear miRNAs in post-transcripitonal processes. To investigate the role of miRNAs in natural immunity against HPV infection, we conducted a genome-wide prediction of potential miRNA targets in four HPV types and found several dozens of potential miRNA binding sites in each HPV genome. We found that HPV16 L1 and E6 expression dramatically increased in Dicer$^{-/-}$ knockout cells compared to Dicer wild-type cells, suggesting that cellular miRNAs might function as a form of natural immunity against HPV gene expression. 15 miRNA seed matches were found in the coding regions of HPV16 L1. A miR-10a binding site in an exonic splicing suppressor (ESS) of the HPV16 L1 was identified for its negative response to endogenous and exogenous miR-10a detectable in the fractionated nucleus by Northern blot. This negative effect by miR-10a on L1 splicing and expression was greatly reduced in highly differentiated keratinocytes where miR-10a expression is dramatically decreased and productive HPV life cycle can be completed. We also demonstrated that disruption of the miR-10a binding site by point mutation promotes L1 RNA splicing and reduces association of L1 RNA with SRSF1 and Ago-2, a major component of RISC (RNA-induced silencing complex) and a nuclear-cytoplasmic shuttling protein in keratinocytes. Consistently, using a miR-10a with compensatory mutations to the mutated miR-10a binding site inhibited L1 RNA splicing. This study provides the first evidence of miRNA involvement in regulation of RNA splicing by direct interaction with RNA cis-elements.

P-123

CRYSTAL STRUCTURE OF "SPINACH", AN RNA MIMIC OF GFP

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Green fluorescent protein (GFP) and its derivatives have revolutionized the study of proteins. Spinach is a recently reported RNA mimic of GFP, which as genetically encoded fusions, makes possible live-cell, real-time imaging of biological RNAs, without resorting to large RNA-binding protein-GFP fusions. To elucidate the molecular basis of Spinach fluorescence, we have solved its co-crystal structure bound to its cognate exogenous chromophore, revealing that Spinach activates the small molecule by immobilizing it between a base triple, a G-quadruplex, and an unpaired guanine. Mutational and NMR analyses show that the G-quadruplex is essential for Spinach fluorescence, is also present in other fluorogenic RNAs, and may represent a general strategy for RNAs to induce fluorescence of chromophores. The crystal structure has guided the design of a miniaturized 'Baby Spinach', and provides the basis for structure-guided design and tuning of fluorescent RNAs.

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P-124

lncRNA DISCOVERY AND FUNCTIONAL SCREENING BY CRISPRi IN B CELL LYMPHOMA

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Diffuse large B cell lymphoma (DLBCL) is an aggressive cancer that hijacks the activation and survival programs of normal B cells to drive tumorigenesis. While many protein-coding genes have been characterized for their role in DLBCL, lncRNAs remain largely unexplored as a source of novel oncogenes or tumor suppressors in this cancer. Here, we present a functional genomics effort to discover and characterize lncRNAs that drive DLBCL growth and survival. De novo assembly, filtering, and differential expression analysis of 301 RNA-seq datasets from primary and cell line DLBCL samples identified a candidate set of 597 lncRNAs. These lncRNA candidates are differentially expressed between activated B cell-like (ABC) and germinal center B cell-like (GCB) DLBCL subtypes, or they are uniquely expressed in DLBCL cells relative to a panel of ENCODE cell types. 353 putatively novel lncRNAs were discovered that pass filtering criteria, with the majority supported by chromatin state maps from DLBCL ChIP-seq and DNase-seq data. 91 lncRNA candidates are located within recurrent amplifications or deletions in DLBCL. CRISPRi has demonstrated robust inhibition of selected lncRNAs, and current efforts aim to systematically screen candidates for viability phenotypes in ABC and GCB DLBCL cell lines. This ongoing work will identify and characterize lncRNAs that are essential for DLBCL and will provide insight into the role of lncRNAs in cancer.

P-125

STRUCTURAL ANALYSIS OF A RIBOSWITCH CLASS THAT BINDS NICKEL OR COBALT IONS TO CONTROL EXPRESSION OF HEAVY METAL TRANSPORTERS

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Bacteria regularly encounter widely varying metal concentrations in their surrounding environment. As metals become depleted, or, conversely, accrue to toxicity, microbes will activate cellular responses that act to maintain metal homeostasis. A suite of metal-sensing regulatory ('metalloregulatory') proteins orchestrate these responses by allosterically coupling the selective binding of target metals to the activity of DNA-binding domains. However, we report here the discovery, validation and structural details of a widespread class of riboswitch RNAs, whose members selectively and tightly bind the low abundance transition metals, Ni^{2+} and Co^{2+}. These riboswitches bind metal cooperatively, and with affinities in the low micromolar range. The structure of a Co^{2+}-bound RNA reveals a network of molecular contacts that explain how it achieves cooperative binding between adjacent sites. These findings reveal that bacteria have evolved to utilize highly selective metalloregulatory riboswitches, in addition to metalloregulatory proteins, for detecting and responding to toxic levels of heavy metals.

P-126

RNA CHARACTERISTICS THAT CONTRIBUTE TO DECAY DURING THE KAPOSI'S SARCOMA ASSOCIATED HERPESVIRUS LYTIC PHASE

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Kaposi's sarcoma-associated herpesvirus (KSHV) is an opportunistic pathogen of HIV patients and the etiological agent of several human cancers, including Kaposi sarcoma and primary effusion lymphoma. The KSHV life cycle includes a latent phase, when viral gene expression is largely absent, and a lytic phase, when the virus selectively degrades host cell mRNAs (a phenomenon known as the host shutoff effect) and produces progeny virions. A single viral protein, known as SOX, can recapitulate the host shutoff effect. Despite this potent host RNA degradation, the virus expresses a 1-kb, non-coding, polyadenylated nuclear RNA called PAN that accumulates to approximately 500,000 copies per cell. The work of Joan Steitz and colleagues has described a triple-helical RNA secondary structure that caps the 3′-end of this viral lncRNA, thereby contributing to the accumulation of PAN RNA. Crystallographic analysis of the PAN ENE with a poly(A) oligonucleotide revealed that the U-rich internal loop of the ENE forms a triple-strand interaction with the poly(A) tail of its own transcript. Characterization of the RNA decay mechanisms present during the lytic phase of the KSHV life cycle, and therefore counteracted by the PAN RNA ENE, has led to new insights into the mechanism by which host RNAs are degraded during the SOX-mediated host shutoff effect. Based on analysis of RNA sequencing data and biochemical assays we have identified several characteristics of host mRNAs that influence their stability in the presence of transiently expressed SOX protein.

P-127

BRAVEHEART, A LONG NON-CODING RNA, REGULATES CARDIOVASCULAR LINEAGE COMMITMENT BY CONSERVED RNA STRUCTURAL MOTIFS

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Long non-coding RNAs (lncRNAs) have emerged as a new cast of players with roles in modulating developmental gene expression programs. Insights into the roles and mechanisms of lncRNAs in regulating cardiac commitment are only beginning to emerge and remain largely unexplored. Our previous work discovered Braveheart (*Bvht)*, the first in a growing class of lncRNAs that function in heart development in mouse. *Bvht* is required for cardiovascular lineage commitment and maintenance of cardiac cell fate in neonatal cardiomyocytes (CMs), however, we currently lack a mechanistic understanding of its mode of action. Here, we experimentally determine the secondary structure of *Bvht* and identify functional motifs by combining RNA chemical probing and CRISPR/Cas9-mediated genomic editing. In addition, we identify proteins that interact with these functional regions and the genomic targets they regulate. Our work characterizes an important new pathway of transcriptional regulation and sheds light on the molecular switches and key control points that mediate heart development. These results also serve as a basis for future studies to determine the function of *Bvht in vivo* as well additional heart-associated lncRNAs.

P-128

ATAXIN-2 STABILIZES mRNA THROUGH THE DIRECT BINDING TO THE DISTINCT MOTIFS IN 3'UTRs

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It has been proposed that Ataxin-2, a member of the LSm protein family, participates in the regulation of RNA metabolism through interaction with PABPC1. Furthermore, abnormal expansion of polyglutamine (polyQ) in the human-specific polyQ domain of Ataxin-2 to more than 34 repeats leads to spinocerebellar ataxia type 2 (SCA2), whereas intermediate polyQ expansion (27–33 repeats) is associated with risk for amyotrophic lateral sclerosis (ALS), suggesting a common pathogenic role for Ataxin-2 in both neurodegenerative diseases. However, the exact biological mechanism and *in vivo* targets of Ataxin-2 remain unknown. Here we report that Ataxin-2 binds directly to RNAs in a PABPC1-independent manner. High-throughput sequencing of Ataxin-2-bound RNAs prepared by PAR-CLIP revealed that Ataxin-2 binds predominantly to uridine-rich elements, including well-characterized cis-regulatory AU-rich elements, in the 3'UTRs of target mRNAs. Gene expression analysis after Ataxin-2 depletion or overexpression revealed that Ataxin-2 stabilizes target mRNAs and increases the abundance of corresponding proteins. This function is similar to that of other RNA-binding proteins, such as HuR. Comparative analysis of the binding-sites of Ataxin-2 and HuR demonstrated that both RNA binding proteins coordinately regulate a subset of genes with each protein binding to distinct U-rich elements at different positions within a given 3'UTR. These findings suggest that Ataxin-2 is an RNA-binding protein that targets cis-regulatory elements in 3'UTRs to stabilize a subset of mRNAs and increase protein expression in coordination with other RNA-binding proteins. Finally, we found that disease-associated polyQ expansion downregulates the physiological activity of Ataxin-2. This evidence indicates that partial loss of Ataxin-2 function might play a causative role in the pathogenesis of neurodegeneration, at least in part.

P-129

COMMON EFFECTS OF ALS-ASSOCIATED MUTATIONS ON RNA LOCALIZATION: A ROLE FOR CYTOPLASMIC RNA INCLUSIONS

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Diverse mutations cause a spectrum of neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Such mutations can affect RNA-binding proteins (such as Fus and TDP-43) and commonly lead to the formation of cytoplasmic inclusions containing either one or the other protein. Formation of Fus- or TDP-43-containing inclusions is a major hallmark of disease, and one hypothesis suggests that they contribute to a toxic gain of function that leads to disease. Another, non-mutually exclusive hypothesis suggests that loss of nuclear functions of Fus and TDP-43, such as in transcription or alternative splicing, are pathogenic

We had previously reported a new cytoplasmic role for Fus. Wild-type Fus associates with ribonucleoprotein (RNP) complexes containing the tumor-suppressor protein Adenomatous Polyposis Coli (APC) and appears to control their translation. These complexes are localized within protrusive areas of cells and are specifically anchored at the plus ends of detyrosinated microtubules (Glu-MTs), i.e. a subset of stable microtubules that do not undergo dynamic instability. Expression of mutant Fus, carrying ALS-associated mutations, leads to recruitment of APC within Fus cytoplasmic granules. We now show that mutant Fus expression leads to mislocalization of at least some APC-associated RNAs from cell protrusions and to a preferential disruption of detyrosinated microtubules. Interestingly, expression of ALS-associated TDP-43 mutants leads to similar disruption of localized RNAs and of the microtubule network. Strikingly, formation of cytoplasmic granules appears to be important, since disrupted phenotypes are observed only in cells exhibiting cytoplasmic Fus granules, but not in cells expressing non-aggregated forms of the mutant protein. Our results indicate that RNA mislocalization is a common effect of pathogenic mutations in Fus and TDP-43, and suggest that it is manifested upon pathogenic protein aggregation. We are investigating the underlying mechanisms and are identifying globally the affected RNAs.

P-130

SIMULTANEOUS DETECTION OF CIRCULATING ONCOmiRs FROM LIQUID BIOPSIES FOR PROSTATE CANCER SCREENING USING GRAPHENE NANODEVICES

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Circulating oncomiRs are highly stable diagnostic, prognostic and therapeutic tumor biomarkers, which can reflect the status of the disease and response to cancer therapy. *miR*-141 is an oncomiR, which is overexpressed in advanced prostate cancer patients, whereas its expression is at the normal levels in the early stages of the disease. On the other hand, *miR*-21 is significantly elevated in the early-stage, but not in the advanced prostate cancer. Here, we have demonstrated simultaneous detection of exogenous *miR*-21 and *miR*-141 from human liquid biopsies including blood, urine and saliva using graphene nanodevices. Our system enables us to specifically and reliably detect each oncomiR at different fluorescence emission channels from a large population of RNAs extracted from body fluids. We have also demonstrated that the sensitivity of our diagnostic nanodevices can be tuned by incorporating a highly specific enzymatic step. The approach in this study combines two emerging fields of nano-graphene in biomedicine and the role of circulating miRNAs in cancer. The reported graphene nanodevice is simple to assemble and operate. The prostate cancer screening approach using liquid biopsies is non- or minimally invasive and can be combined with the current diagnostic methods to minimize low selectivity and sensitivity. Our approach also enables us to identify the aggressiveness of the disease. Therefore, the strategy reported here has the potential to address the current challenges in diagnosis, prognosis and staging of prostate cancer with a non- or minimally invasive approach.

P-131

microRNA TRANSFERASE FUNCTION OF AUF1 p37

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Eukaryotic gene expression is tightly regulated posttranscriptionally by RNA-binding proteins (RBPs) and microRNAs. Silencing the RBP AU-rich-binding factor (AUF)1 reduced the interaction of microRNAs with Argonaute 2 (Ago2), the catalytic component of the RNA-induced silencing complex (RISC). Analysis of this effect revealed that the AUF1 isoform p37 displayed high affinity for the microRNA let-7b, promoted the interaction of let-7b with Ago2, and enhanced Ago2-let-7-mediated mRNA decay. Our findings uncover a novel mechanism whereby microRNA transfer from AUF1 p37 to Ago2 facilitates microRNA-elicited gene silencing.

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TARGETING SINGLE BETA-ACTIN mRNA TO SYNAPSES

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Localization of mRNA provides a strategy for cells to regulate protein synthesis within discrete compartments. In neurons, mRNA transport and translation at synapses would obviate the need to procure plasticity-related proteins from the soma. In dendrites, single b-actin mRNAs can move, suggesting that individual synapses could recruit mRNA when stimulated. However, this has never been shown. To determine whether b-actin mRNAs could be trafficked to activated synaptic spines, we delivered local stimulation to dendritic spines by diffraction-limited glutamate uncaging and imaged the appearance of mRNAs at this site. We show that endogenous b-actin mRNAs localized to the base of stimulated spines within 10 minutes after uncaging and remained anchored for more than 120 minutes. This localization required the activity of NMDA receptors and a dynamic actin cytoskeleton, providing a link between structural plasticity of spines and mRNA capture. Inhibition of translation had no effect on localization indicating that dendritic control of mRNA trafficking and local translation was decoupled. Thus our results demonstrate that stimulated dendritic spines can capture b-actin mRNAs and suggest that synaptic activity can recruit the requisite mRNAs to undergo long-lasting structural plasticity.

P-133

PROBING THE MECHANISM OF ATP UTILIZATION IN mRNA RECRUITMENT TO THE EUKARYOTIC RIBOSOME

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Perturbation of accurate protein synthesis plays a role in developmental defects, neurodegenerative diseases, cancer, and viral infections. Most of translational control is achieved during the initiation phase. In eukaryotes, a dozen Initiation Factors (eIF) form a pre-initiation complex (PIC) with the small subunit of the ribosome (40S) and an mRNA. Eukaryotic translation Initiation Factor 4A (eIF4A) is an RNA-dependent ATPase and RNA helicase, thought to remove mRNA structure and promote mRNA recruitment to the PIC. The enzyme is well characterized in isolation, but the mechanism of how it promotes mRNA recruitment in the context of the PIC is not clear. Using an *in vitro* reconstituted *S. cerevisiae* translation initiation system we are trying to understand how eIF4A utilizes ATP and contributes to recruitment of mRNAs to the ribosome. Rates of eIF4A ATPase were compared in the absence and presence of various components of initiation machinery as well as the entire PIC. In addition, we are able to follow a labeled mRNA during recruitment to the ribosome via a gel shift assay. Our preliminary data suggest that the activity and mechanism of eIF4A during mRNA recruitment to the PIC may be distinct from what is observed when eIF4A acts on RNA substrates and ATP in isolation.

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PANORAMIX ENFORCES piRNA-DEPENDENT CO-TRANSCRIPTIONAL SILENCING

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Piwi proteins and its associated small RNAs are essential for animal fertility in animals. In part, this is due to their roles in guarding germ cell genomes against transposons. The nuclear Piwi protein is indispensable for the transcriptional repression of transposons in both germ and somatic follicle cells of *Drosophila* ovary. We and others have shown that Piwi-mediated transcriptional silencing is correlated with the deposition of heterochromatic marks H3K9me3 over transposons. However, neither do we understand the mechanism of silencing nor is the identity of the histone methyltransferase known.

To address these questions, we took advantage of a λN /BoxB tethering system to mimic piRNA targeting. Surprisingly, artificial tethering of λN-Piwi to a luciferase reporter containing BoxB sites failed to silence the expression of the reporter. Moreover, the reporter remained actively transcribed even when Piwi is tethered simultaneously with another piRNA silencing effector, Asterix/DmGTSF1. This is in direct contrast with the observation that guiding Piwi complexes via artificial piRNAs can lead to repression of their complementary targets. Taken together, our data suggest that additional factor(s) and/or conformation changes are required to license Piwi complexes to repress transcription.

Through mining the data from several independent genome-wide RNAi screens for factors required for transposon silencing, we identified a novel protein-coding gene that can influence global transposon expression in a similar fashion as Piwi when knocked down by RNAi. The effect is not due to the defects of piRNA biogenesis since levels of piRNAs remained unchanged and Piwi maintained its nuclear localization. Strikingly, enforced tethering of this protein to nascent mRNA transcripts causes co-transcriptional silencing of the source locus and the deposition of repressive chromatin marks. Because of its function in the piRNA pathway and relationship to Asterix, we have named this protein Panoramix, the mentor who empowers Asterix to perform his feats of strength. Importantly, we found that both Eggless, an H3K9 methyltransferase, and HP1a, an H3K9me2/3 binder, are required for Panoramix-mediated transcriptional silencing. Finally, we demonstrated that Panoramix binds to genomic loci that harbor transposon sequences in a manner that depends upon Asterix. We propose that Panoramix forms one of the missing links between the piRNA pathway and the general silencing machinery that it recruits to enforce transcriptional repression to protect germline from deleterious transposons.

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IDENTIFICATION OF microRNAs IN BLOOD AND URINE AS POTENTIAL BIOMARKERS FOR PROSTATE CANCER DETECTION

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MicroRNAs (miRNA) are small non-coding RNAs that are very important in post-transcriptional regulation. MiRs bind mostly to the 3'-UTR of mRNAs to negatively regulate gene expression at the level of translation. Previous research has shown that miRNAs may either serve as tumor suppressors or oncomirs dependent on what role the genes they target play in cancer progression. Interestingly, miRNAs can be secreted in a variety of protective capsules and as such can act as cellular transduction signals affecting gene expression at secondary sites. The aberrant expression of some miRNAs in patient blood and or urine may be diagnostic for specific cancers and/or even contribute to tumorigenesis. Thus, miRNAs can be relevant biomarkers for identifying and staging cancer. Our laboratory has been focusing on identifying miRNAs in blood and urine as potential biomarkers for prostate cancer (pCa), the second leading cause of cancer death in men in the United States. In this study, we use Illumina ® Next Generation Sequencing to generate miRNA profiles in samples from pCa patients compared to normal individuals. Analysis of deep sequencing reads was conducted through Partek Flow, v3.0 software using Bowtie 2 (v2.1.0) short read aligner with miRBase v. 20 as the reference database for alignment and annotation. An average of 85% of total reads after trimming aligned to the miRbase and the average quality of each read was 38.3 where a value above 30 is thought to be relevant. According to the deep sequencing data, a number of miRNAs are significantly up-regulated or down-regulated. Quantitative real-time PCR (qRT-PCR) is being performed on selected miRNAs to confirm the deep sequencing analysis. Ultimately, it is anticipated that the results from this study will provide considerable insight into improving current pCa diagnosis by offering an alternative method for early detection and progression, since current monitoring of PSA levels has led to many false positive and over treatments with dire outcomes.

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STRUCTURE AND MECHANISM OF THE T-BOX RIBOSWITCH, BACTERIAL SENSORS OF AMINO ACID STARVATION

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Bacterial T-box riboswitches sense intracellular amino acid availability by directly binding tRNAs and monitoring their aminoacylation status. It remains unknown how the T-box recognizes its cognate tRNA, detects its aminoacylation state, and directs genetic switching. We define the T-box Stem I as the minimal domain necessary and sufficient for specific, high-affinity tRNA binding, and report its co-crystal structure complexed with cognate tRNA at 3.2 Å resolution. The C-shaped Stem I cradles the L-shaped tRNA forming an extended interface, simultaneously recognizing the anticodon and elbow of tRNA. There are noteworthy structural parallels with the ribosome and RNase P. Using chemical analogs, we find that the T-box detects the molecular volume of tRNA 3'-substituents. Combined calorimetric and fluorescence lifetime analyses demonstrate that this discrimination results from intimate, coaxial stacking between the tRNA acceptor end and a helix in the T-box 3' domain. This intermolecular stacking, selective for uncharged tRNA, stabilizes the antiterminator and allows transcription readthrough into downstream coding regions.

Taken together, the T-box paradigm demonstrates that non-coding RNAs can recognize each other not just through sequence complementarity, but through structural specificity using mutually induced fit between precisely positioned RNA motifs. It also provides a proof of principle that compact RNA domains can sense minute chemical changes on another RNA (such as tRNA aminoacylation), and alter gene expression based on that readout. These findings hint at the possible existence of networks of interconnected non-coding RNAs and a potential for RNA devices to directly read and act on epigenetic marks.

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PRIMATE-SPECIFIC miR-576-3P SETS HOST DEFENSE SIGNALLING THRESHOLD

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MicroRNAs (miRNAs) have been shown to regulate viral infection, but the miRNAs that target intracellular sensors and adaptors of innate immunity have not been fully uncovered. Here we conduct an miRNA mimic screen and validation with miRNA inhibitors in cells infected with vesicular stomatitis virus (VSV) to identify miRNAs that regulate viral-host interactions. We identify miR-576-3p as a robust regulator of infection by VSV and other RNA and DNA viruses. While an miR-576-3p mimic sensitizes cells to viral replication, inhibition of endogenous miR-576-3p prevents infection. miR-576-3p is induced by IRF3 concomitantly with interferon and targets STING, MAVS and TRAF3, which are critical factors for interferon expression. Interestingly, miR-576-3p and its binding sites are primate-specific and miR-576-3p levels are reduced in inflammatory diseases. These findings indicate that induction of miR-576-3p by IRF3 triggers a feedback mechanism to reduce interferon expression and set an antiviral response threshold to likely avoid excessive inflammation.

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RNA TOPOISOMERASES: THEIR PREVALENCE AND ROLES IN NEURODEVELOPMENT AND MENTAL DISORDERS

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Topoisomerases can catalyze DNA strand passage reactions, and are known to solve topological problems of DNA during replication, repair, and transcription. Interestingly, several members of the type IA topoisomerase family, Top3 from E. coli (Wang et al. PNAS, 1996) and Top3β from human (Xu et al. Nat. Neurosci., 2013), are also capable of catalyzing RNA strand passage reactions. However, the prevalence and function of RNA topoisomerases are unclear. We have previously shown that Top3b forms a stoichiometric complex with TDRD3; and this complex interacts with FMRP, a protein deficient in Fragile X syndrome and known to regulate translation of mRNAs important for neuronal function and autism spectrum disorder (ASD). Top3b mutation in mice and flies results in abnormal synaptic gene expression as well as synapse formation (Xu et al. Nat. Neurosci., 2013), whereas its deletion in humans has been linked to schizophrenia and intellectual disability (Stoll et al., Nat. Neurosci., 2013). Here we have further investigated the prevalence and function of RNA topoisomerases, and made the following discoveries. One, several Type IA topoisomerases from bacteria (*Thermatoga maritima*), archaea (*Nanoarchaeum equitans*), and yeast (*Sacchromyces cerevisiae*) are capable of catalyzing RNA strand passage reactions, similar to their homologs in E. coli and human. Two, the catalytic Tyr residue essential for the DNA topoisomerase activity is also required for the RNA topoisomerase activity, suggesting that similar mechanisms are used for both substrates. Three, the unique C-terminal Zn-fingers and RGG RNA binding domain of human Top3β are required for its RNA topoisomerase activity. Fourth, Drosophila Top3β mutants that lacks the RGG domain or has the catalytic Tyr residue substituted by Phe are unable to rescue the defective synapse formation in flies with Top3b deletion. Finally, two de novo single nucleotide variants of Top3β identified previously in schizophrenia and ASDs are compromised in either FMRP association, or RNA topoisomerase activity, or both. These data suggest that RNA topoisomerases are present in all three domains of life (bacteria, eukaryotes, and archaea), and are important for promote neurodevelopment and prevent mental dysfunction in human.

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LONG NON-CODING RNA PVT1 AUGMENTS MYC IN HUMAN CANCER

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Though gain of 8q24.21 is a common mutation in many human cancers, its functional annotation has been limited to studying myelocytomatosis (MYC), the prominent oncogene that maps in the gained region. However, MYC is often co-gained with an adjacent 'gene desert' region, encompassing the long non-coding RNA gene Plasmacytoma variant translocation 1 (PVT1), the CCDC26 gene candidate and gasdermin C GSDMC. Whether copy number gain of one or more of these genes drives neoplasia is not known. We used chromosome engineering in mouse embryonic stem cells to develop strains with an extra copy of 1) Myc, 2) Pvt1, Ccdc26, Gsdmc, and 3) Myc, Pvt1, Ccdc26, Gsdmc. When rat Neu was introduced into these three strains to test the change in the latency and penetrance in mammary tumor development, only the mice with an extra copy of Myc, Pvt1, Ccdc26, Gsdmc (but not those with an extra copy of Myc or Pvt1, Ccdc26, Gsdmc)developed adenocarcinomas with reduced latency and higher penetrance, suggesting that while an extra copy of Myc gene failed to measurably advance cancer, it may co-operate with Pvt1, Ccdc26 or Gsdmc to promote cancer. Si-RNA mediated knockdown of Pvt1/PVT1 reduced the proliferation rates in the mouse mammary tumors, as well as two human breast cancer cell lines with 8q24 amplification (SK-BR-3 and MDA-MB-231). Ablation of PVT1 markedly decreased MYC protein levels, while no effect was seen in MYC transcript levels, suggesting a PVT1-dependence of MYC protein in MYC amplified cancer cells. Analysis of the cancer genome atlas and Progenetix databases suggested that 18% of 45,922 human tumors queried harbor MYC copy number increases, and in >99% of these cancers, PVT1 copy number is co-increased. Tissue microarray analysis of in situ hybridization for PVT1 and immunohistochemistry for MYC revealed that PVT1 RNA and MYC protein expression are correlated in primary human tumors. These data together suggests that PVT1 can potentiate MYC in human cancers. CRISPR mediated deletion of PVT1 in colorectal cell line HCT116 either ablated tumor formation in xenografts, and significantly reduced MYC protein levels. Further studies reveal the oncogenic potential of PVT1 in cancers even without supernumerary 8q24. We propose that the dependence of high levels of MYC on lncRNA PVT1 provides a much-needed therapeutic target against MYC protein, which has been refractory to small molecule inhibition.

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INHIBITION OF THE SPLICING OF THE *EWS-FLI* FUSION TRANSCRIPT REVERSES EWS-FLI DRIVEN ONCOGENIC EXPRESSION IN EWING SARCOMA

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Ewing sarcoma (ES) is a highly aggressive cancer of the bone and soft tissue. In ~85% of ES tumors the primary oncogenic event is a t(11:22)(q24:q12) translocation that generates a fusion of the 5' end of *EWSR1* and the 3' end of *FLI1* referred to as *EWS-FLI1*. The exact genomic breakpoints within the *EWSR1* and *FLI1* genes vary, but typically occur within introns and require the splicing machinery to generate an in-frame *EWS-FLI1* transcript. The most common *EWS-FLI1* transcripts fuse either exon 7 of *EWSR1* to exon 6 of *FLI1* (a type I or a 7/6 fusion), or fuse exon 7 of *EWSR1* to exon 5 of *FLI (*a type II or 7/5 fusion). In an estimated 40% of EWS-FLI1 driven tumors the generation of an in-frame *EWS-FLI1* fusion transcript requires alternative splicing. In particular, translocations that retain exon 8 of *EWSR1* generate an out-of-frame transcript unless this exon is removed. Using an assay of EWS-FLI1 activity and genome-wide siRNA screening we have identified RNA processing as a therapeutic vulnerability in ES.

Parallel genome-wide siRNA-mediated RNAi screens were conducted in ES TC32 cell lines expressing a luciferase (luc) reporter protein driven by either the promoter of the EWS-FLI1 target gene *NR0B1* (TC32-NR0B1-luc) or the CMV promoter (TC32-CMV-luc). The top gene ontology terms associated with the 28 priority candidate genes that when silenced induced a differential decrease in the TC32-NR0B1-luc signal versus the TC32-CMV-luc signal were mRNA splicing (p-value = $1.42E^{-08}$) and mRNA processing (p-value = $2.32E^{-07}$). To investigate the mechanistic basis for the identification of specific RNA processing proteins as required for the activity of EWS-FLI1 we focused on two lead candidate genes, the heterogeneous nuclear ribonucleoprotein H1, *HNRNPH1*, and the core splicing factor, *SF3B1*. Using PCR analysis we determined that HNRNPH1 is required for the splicing of *EWS-FLI1* fusion transcripts expressed in ES cells in which the chromosome 22 breakpoint retains *EWSR1* exon 8, specifically in TC32 and SKNMC ES cells. We also show ES cell lines harboring 7/ 6 (TC32, SKNMC, and TC71) or 7/ 5 (RD-ES) *EWS-FLI1* fusions are all sensitive to the loss-of-function of SF3B1. Quantitative RT-PCR, immunoblot, and whole transcriptome analysis show that disrupted splicing of the *EWS-FLI1* transcript alters its expression and reverses the expression of a significant proportion of genes that are targets of EWS-FLI1. These observations were confirmed in four ES cell lines using the splicing inhibitor, Pladienolide B.

Our results provide the basis for a novel strategy to target fusion oncogenes by interfering with RNA processing. This study has implications for the treatment of ES through inhibition of proteins required for expression of the *EWS-FLI1* transcript and identifies a candidate compound for further clinical development. Our findings may also open up strategies for treatment of other cancers driven by fusion oncogenes.

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A NOVEL ROLE FOR 3'UTR SEQUENCES IN REGULATING THE ACTIVITY OF THE C-MYC ONCOPROTEIN

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The 3' untranslated regions (3'UTRs) of mRNAs play essential roles in regulating the stability, polyadenylation status, nuclear export, localization and expression levels of the message and the encoded protein. Recently, our laboratory showed that the 3'UTR of the *Cebpb* mRNA, which encodes an autorepressed transcription factor activated by oncogenic Ras signaling, inhibits Ras-induced posttranslational activation of the translated protein and consequently suppresses C/EBPb activity in immortalized and transformed cells. This mechanism involves 3'UTR-mediated targeting of the *Cebpb* mRNA to a peripheral region of the cytoplasm.

Here, we have investigated the role of 3'UTR sequences in regulating the activity of the c-Myc protein in immortalized NIH 3T3 cells. c-Myc is a potent oncogenic transcription factor that plays an indispensable role in cellular growth, proliferation, metabolism and transformation. We used expression constructs containing only the c-Myc coding region (Myc^{CR}) or coding region plus the 3'UTR (Myc^{UTR}) to investigate whether the 3'UTR affects the proliferation, anchorage independent growth and transcriptional activating functions of c-Myc. In addition, the subcellular localization of the transcripts was investigated using confocal microscopy. We show that the 3'UTR enhances the proliferative and transformation-stimulating functions of c-Myc in Ras-transformed NIH 3T3 cells without significantly affecting protein expression. In addition, the 3'UTR enhances the transcriptional activity of c-Myc on the prothymosin a promoter, a transcriptional target of c-Myc. Furthermore, mRNA localization studies revealed the 3'UTR containing transcript is densely localized in a perinuclear cytoplasmic domain, whereas transcripts lacking the 3'UTR exhibit a diffuse cytoplasmic distribution. Of note, in primary cells (MEFs) both transcripts showed uniformly diffuse cytoplasmic distribution. Our data suggest that in immortalized cells the 3'UTR localizes Myc transcripts to a perinuclear cytoplasmic region where c-Myc activating kinases reside. In summary, our results indicate that the 3'UTR stimulates c-Myc activity, thus identifying a previously unknown mechanism regulating oncogenicity of the c-Myc protein through mRNA compartmentalization.

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ACTIVATION OF THE INNATE IMMUNE SENSOR, PKR BY THREE CLASSES OF BACTERIAL RIBOSWITCHES

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The innate immune system is the first line of defense against invading pathogens and it is known for its ability to recognize non-specific patterns among these pathogens in order to protect the cell in a generalized way. The RNA-activated protein kinase, PKR is a dsRNA binding protein and an essential sensor in the innate immune response. Upon binding of long dsRNA this protein will autophosphorylate and phosphorylate the eukaryotic initiation factor 2a (eIF2a), which ultimately leads to termination of translation and apoptosis. PKR has a well-characterized role in recognizing viral RNA and is known to bind long stretches (> 33 bp) of dsRNA non-sequence specifically to promote activation. In recent years PKR has been shown to be more permissive, binding other functional and non-conventional RNAs, as well as some proteins. More recently PKR has been shown to be activated by additional pathogens including bacteria. The goal of this research is to characterize how PKR recognizes and interacts with bacterial RNA. We are currently investigating how PKR interacts with representative discrete functional bacterial RNAs and chose three model system riboswitch classes in order to carry out this study. We will present our results on these systems and describe effects of biological Mg^{2+} conditions on activation by specific RNAs.

Nallagatla, SR., Toroney, R. & Bevilacqua, PC., *Curr Opin Struct Biol*, **2011**, *21*, 119-127.
Bleiblo, F., Michael, P., Brabant, D., Ramana, CV., Tai, TC., Saleh, M., Parrillo, J. Kumar, A. & Kumar, A. *J Thorac Dis,* **2012**, *4 (2)*, 114-125.
Bleiblo, F., Michael, P., Brabant, D., Ramana, CV., Tai, TC., Saleh, M., Parrillo, J. Kumar, A. & Kumar, A. *Int J Clin Exp Med*, **2013**, *6 (1)*, 16-25.
Truong, D. M., Sidote, D. J., Russell, R. & Lambowitz, A. M. *Proc. Natl. Acad. Sci.* **2013**, *110,* E3800-3809.

P-143

DIVERGENT TARGETING BY CO-EXPRESSED miR-142-3P 5'-VARIANTS AND SELECTIVE VIRAL MIMICRY

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Sequence heterogeneity at the ends of mature microRNAs (miRNAs) is well documented, but its effects on miRNA function are largely unexplored. Here we studied the impact of miRNA 5'-heterogeneity, which affects the seed region critical for target recognition. Using the example of miR-142-3p, an emerging regulator of the hematopoietic lineage in vertebrates, we show that naturally co-expressed 5'-variants (5'-isomiRs) can recognize largely distinct sets of binding sites. Despite this, both miR-142-3p isomiRs regulate exclusive and shared targets involved in actin dynamics. Thus, 5'-heterogeneity can substantially broaden and enhance regulation of one pathway. Other 5'-isomiRs, in contrast, recognize largely overlapping sets of binding sites. This is exemplified by two herpesviral 5'-isomiRs that selectively mimic one of the miR-142-3p 5'-isomiRs. We propose that other cellular and viral 5'-isomiRs can similarly be grouped into those with divergent or convergent target repertoires, based on 5'-sequence features. Taken together, our results demonstrate that miRNA 5'-end variation leads to differential targeting and thus have profound implications for the function of several miRNAs.

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GLOBAL CHANGES IN TRANSCRIPTION AND RNA PROCESSING DURING SHORT TELOMERE-INDUCED SENESCENCE IN YEAST IDENTIFIED BY RNA-SEQ

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Loss of telomerase activity results in the progressive shortening of telomeric DNA and eventually a specific G2/M cell-cycle arrest known as senescence. In yeast, a small subset of cells known as survivors can escape this arrest by initiating a recombination-mediated telomere lengthening pathway. Using a telomerase-negative yeast strain, we have taken several approaches to elucidate the adaptive changes required in senescent and post-senescent cells, including whole-genome sequencing to identify any potential causative mutations as well as RNA-seq to monitor changes in gene expression. We find no mutations correlating with survivor cells and conclude that genetic changes are not a required step in survivor formation. Our transcriptome data reveal several interesting features of the cellular response to telomerase deletion. First, a shared subset of genes shows differential expression at every time point, consistent with previous reports of a telomerase-deletion response. This subset is particularly enriched for genes involved in amino-acid biosynthesis. Second, both the pre-senescent and survivor samples exhibit widespread down-regulation of ribosomal proteins, some of which also have lower expression in cancer cells. Third, in senescing cells we observe differential expression of ~1100 genes. The differentially expressed genes show significant overlap with changes observed during slow growth and the global starvation response, including upregulation of several key autophagy genes and cell-wall components as well as apparent changes in hexose transporter expression. Further bioinformatic analysis revealed a set of 573 genes that are differentially expressed during senescence, but not during the DNA-damage response, slow growth or in G2/M-arrested cells. This indicates that telomere-induced senescence represents a specific and distinct quiescence-like state. The senescence state is characterized by concerted changes in both the meiotic and ribosome biogenesis machinery. Interestingly, we also observe changes in RNA splicing efficiency as well as an overall increase in transcripts mapping to novel loci, suggesting the appearance of some previously uncharacterized ncRNAs. In our future work, we aim to fully characterize the changes in gene expression and RNA processing associated with short telomere-induced senescence in yeast.

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CIRCULAR INTRONIC RNA IN THE CYTOPLASM OF *XENOPUS* OOCYTES

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Introns represent a significant portion of most eukaryotic genomes, yet very little is known about intronic transcript functions. Intronic lariats are normally produced as intermediates of splicing and are rapidly degraded in the nucleus by the debranching enzyme and by exonucleases. However, a specific set of circular intronic RNAs (ciRNAs) show high stability in the nucleus of both somatic and germ cells. It has been proposed that ciRNAs can regulate transcription of their cognate genes.
By analyzing the cytoplasmic RNA pool of *Xenopus* oocytes, we detected stable intronic sequences. We further demonstrated that these intronic sequences are resistant to RNaseR, a processive 3' to 5' exonuclease. The presence of inverted reads within our RNAseq data confirmed that cytoplasmic intronic sequences are stabilized as circular molecules. The presence of ciRNAs in the cytoplasm implies that they may play other important roles in the cell in addition to transcription regulation. We observed that ciRNAs accumulate during later stages of oogenesis and persist in the early embryo. These observations suggest that ciRNAs could be important maternal factors for early embryogenesis.
To study cytoplasmic ciRNAs, we developed a system to express ectopic ciRNAs independently of their endogenous cognate mRNA *in vivo*. The system we developed will allow us to test potential stability signals within the intronic sequences and perform pulldown assays. Ultimately, we will assess the potential role of cytoplasmic ciRNAs in translation control and mRNA stabilization.

P-146

RNA-DNA SEQUENCE DIFFERENCES IN *SACCHAROMYCES CEREVISIAE*

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Processes that alter mRNA transcript sequences and structures, such as RNA splicing and editing, allows a DNA template to produce two or more RNA transcripts. We and others previously reported widespread RNA editing and other types of RNA-DNA sequence differences (RDD) in human cells. RNA-editing is mediated by deaminases including ADARs and APOBEC whereas mechanisms underlying other types of RDDs are unknown.

In yeast, it was thought that RNA editing only occurs in tRNAs. In this study, we studied RNA-DNA sequence differences in yeast and examined possible mechanisms using mutant strains. We found that like human cells, *Saccharomyces cerevisiae* have all 12 types of RDDs in the mRNA. These sequence differences are propagated to proteins as we identified peptides encoded by the RNA sequences that differ from their DNA templates at RDD sites. A screen of yeast mutants showed that known deaminases do not contribute to yeast RDDs. Instead RDD formation is affected by mutations in genes regulating R-loops, one type of RNA-DNA hybrids. Mutation in genes for ribonuclease H, senataxin and topoisomerase I, which resolve RNA-DNA hybrids through different mechanisms, leads to an increase in RDD frequency and levels.

In summary, our results showed that RDD is a conserved process that diversifies transcriptomes and proteomes.

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YEAST TELOMERASE RNP: EXCEPTIONAL FLEXIBILITY AS WELL AS NEW ESSENTIAL STRUCTURAL FEATURES OF ITS LONG NON-CODING RNA

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The RNA-protein complex telomerase — which permits complete genome replication at chromosome ends to avoid senescence — is aberrantly upregulated in 90% of cancers, while reduction in its function is linked to premature aging and a growing list of telomere syndromes that tend to cause organ failure. At its core, telomerase is composed of a reverse transcriptase (TERT) and a long non-coding RNA. A short segment of the RNA provides "template" nucleotides that TERT copies during telomeric DNA-repeat synthesis. However, the large RNA subunit is much more than just a template. First, it has active roles in catalytic mechanism, including the two essential structures we have recently discovered in the 1157-nt yeast telomerase RNA, TLC1: the Area of Required Connectivity (ARC) in the core and the Second Essential Est1-arm Domain. The ARC connects the four conserved secondary structure elements to permit catalytic activity. Circular permutation analysis that led to discovery of the ARC also showed that the ends of the RNA could be moved to other locations within the core with retention of function *in vitro* and *in vivo*, showing that functional flexibility exists in certain parts of the core (Mefford *et al*., *EMBO* 2013). These locations are also physically flexible junctions, based on recent assaying of each nucleotide's chemical reactivity by SHAPE (Niederer and Zappulla, *RNA* 2015).

In addition to having critical roles in catalysis, telomerase RNA is also required to form the RNP enzyme by providing a scaffold for the protein subunits. We have previously determined that the TLC1 lncRNA tolerates the following perturbations without causing senescence: (1) relocation of the position for each of the enzyme's holoenzyme-specific RNA-binding proteins, (2) deletion of the bulky, rapidly evolving portions of its three long arms, and (3) stiffening of the arms by conversion to uninterrupted double-stranded RNA by deletion of their loops and bulges. With respect to #1 above, relocation of the essential Est1 protein subunits binding site was tolerated at three different positions in TLC1. Recently, we showed that Est1 could even be artificially tethered to TLC1 through a heterologous RNA-protein interaction module (Lebo *et al*., *RNA* – In Press). However, this analysis also led to the discovery that there is a Second Essential Est1-arm Domain (SEED) in a highly conserved 108-nt region around where Est1 binds. The SEED is required for telomerase action after the enzyme is recruited to the telomere. At the telomere, it works Est1-independently and can even function *in trans*. Thus, the SEED has a function beyond scaffolding and may be required for establishing telomere extendibility or promoting telomerase RNP holoenzyme activity. As for perturbations #2 and #3 in TLC1 RNA listed above, these alterations actually improve *in-vitro* telomerase activity compared to wild-type TLC1. This is presumably due to an increased fraction of the RNA molecules forming a natively folded catalytic core. However, both the truncated and stiffened-arm RNAs have reduced length and structure of the long arms that perform the flexible scaffolding role. To create a TLC1 RNA with wild-type length and structure that — unlike WT TLC1 — provides robust *in vitro* activity, we mutated A-U and G-U residues in the long arms to G-C in an effort to promote favorability of native-state folding. We computationally modeled these new "determined-arm" (DA-TLC1) alleles to help with synthetic design. *In vivo*, DA-TLC1 maintains telomeres that are nearly wild-type length and, *in vitro*, DA-TLC1 reconstitutes robust telomerase activity. Thus, by synthetically designing telomerase RNAs to improve the free energy of its folding, we have promoted formation of the phylogenetically supported native structure *in vitro*. DA-TLC1 will facilitate studying structure and function of telomerase and this approach may be useful for examining other RNAs as well.

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